# Supporting Information

## Allosteric indole amide inhibitors of p97: Identification of a novel probe of the ubiquitin pathway

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**Biological Methods**

*Protein Expression and Purification*

Full-length human p97 (residues 1-806), the p97 D2 domain (residues 461-806), along with C-terminal AviTagged full-length p97 and N-terminal AviTagged ND1 domains (residues 1-458) were cloned as previously reported.[1] The C522A mutation was introduced into full-length p97 using PCR-mediated site directed mutagenesis. All constructs were verified by DNA sequencing.

Recombinant full-length p97 and D2 domain were expressed in *E. coli* Rosetta 2(DE3) by inducing with 0.1 mM IPTG and growing at 25 °C for 16 hours. To generate biotinylated proteins for SPR, AviTagged p97 constructs were expressed as previously described.[1] All proteins were purified using a combination of Ni-NTA affinity purification, TEV cleavage, and size exclusion chromatography (Superdex 200 16/60) in 25 mM Tris pH 7.8, 150 mM NaCl, 0.5 mM Tris-(2-carboxyethyl) phosphine (TCEP).[1] For 1D NMR experiments, PBS supplemented with 0.5 mM TCEP was used for the size exclusion chromatography.

*Biochemical Assay*

The activity of p97 was measured using the ADP-Glo™ Max assay (Promega V7002) according to manufacturer's protocol.[1] Compounds or a DMSO control (50 nL) were pinned into white, low volume 384-well plates (Corning) containing 5 µL of ATP substrate (40 µM or 200 µM) in assay buffer (10 mM Tris, pH 7.4, 20 mM MgCl2, 1 mM EDTA, 0.5 mM TCEP, 0.01% Triton X-100). p97 (5 µL at 40 nM) was then dispensed into each well to make the final assay conditions 20 µM or 100 µM ATP and 20 nM p97 in assay buffer. The reaction was incubated for 90 min at 22 °C. Luminescence was measured on an Envision multi-mode plate reader (PerkinElmer) and was converted to % inhibition relative to the average negative (0% inhibition; DMSO) and positive (100% inhibition; no ATP) controls within the plate using Equation 1.

[1] Chou, T.-F.; Bulfer, S. L.; Weihl, C. C.; Li, K.; Lis, L. G.; Walters, M. A.; Schoenen, F. J.; Lin, H. J.; Deshaies, R. J.; Arkin, M. R., "Specific inhibition of p97/VCP atpase and kinetic analysis demonstrate

Equation 1: % inhibition = (average $Lum_{neg} - Lum_{well}$)/(average $Lum_{neg} - Lum_{pos}$) x 100.

$IC_{50}$ values were calculated from duplicate or triplicate data by transforming the [compound] to log[compound] and fitting a four-parameter (top set to 100, and bottom set to 0) sigmoidal dose response equation in Prism (GraphPad software).

To determine the mechanism of inhibition, $IC_{50}$ values of **SMDC818909** were determined at 100 μM ATP, 20 μM ATP and 5 μM ATP final concentrations. In addition, the inhibition pattern and inhibition constant were determined for **3** by varying the concentration of ATP (0-400 μM) at various fixed concentrations of compound. To determine $K_i$ values, the Michaelis-Menten equation for a mixed inhibitor (Equation 2) was globally fit to the data.

Equation 2: $v_o = V_{max}[S]/(K_m(1 + [I]/K_i) + [S](1+ [I]\ K'_i))$

where $K_i$ and $K'_i$ represent the equilibrium inhibition constants for competitive and uncompetitive inhibition, respectively.

*Surface Plasmon Resonance*

Surface plasmon resonance experiments were conducted on a Biacore 4000 instrument (GE healthcare). Biotinylated full-length p97 and the ND1 domains were immobilized to 3000-5000 response units (RUs) on CM5 sensor chips as previously described.[1] Compound binding (0-67.5 μM; 2-fold dilutions) was measured at 20 °C in 25 mM Tris, pH 7.5, 150 mM NaCl, 10 mM $MgCl_2$, 0.5 mM TCEP, 0.005% Tween-20, 5% DMSO.

*Nuclear Magnetic Resonance*

All 1D NMR spectra were acquired on a Bruker AVANCE DRX500 MHz spectrometer at 296.8 K (referenced to 4% $CH_3OH/CH_3OD$ using a coefficient of 1.0183) with a 5 mm Bruker QCI Cryoprobe ($^1H$, $^{13}C$, $^{15}N$ & $^{31}P$) with actively shielded Z-gradients (Bruker Biospin Corp., Billerica MA). Samples (500 μL) containing 100 μM compound and 5 or

10 μM D2 protein were prepared in Norell XR-55 glass NMR tubes in 100% $D_2O$ PBS (pH 7.5), 1 mM $NaN_3$ and 1 mM DTT, 0.1% DMSO. The 'zggpw5' pulse program from the Bruker pulse program library (TopSpin 1.3 pl10) was used for data acquisition with an excitation sculpting double gradient echo-based 3-9-19 composite pulse WATERGATE-5 element for water suppression. Topspin 3.2 (Bruker Biospin, Billerica, MA) was used for data analysis.

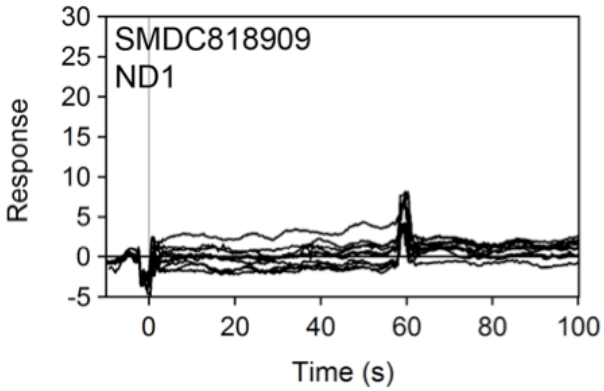


**Figure S1. SPR sensorgrams for SMDC818909 binding to the ND1 domains (residues 1-458) of p97**. The same concentrations of compound (0-62.5 μM) were assayed by SPR against both full-length p97 (Figure 2b) and the ND1 truncation lacking the D2 domain. No binding was observed to the ND1 domains indicating that the D2 domain is the likely binding site for SMDC818909.

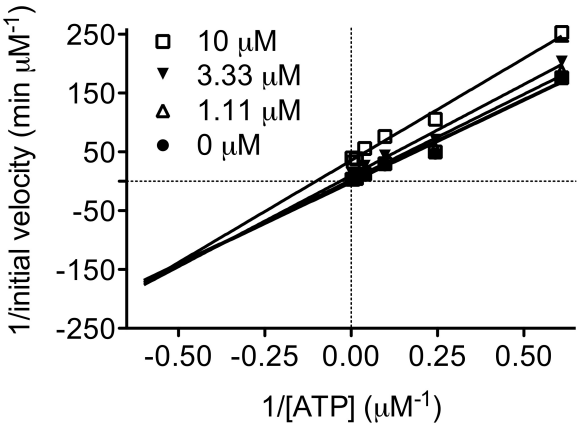


**Figure S2. Extended Lineweaver-Burk plot for UPCDC30005 inhibition of p97.** Plot lines intersect in the lower-left quadrant consistent with a mixed model of inhibition.

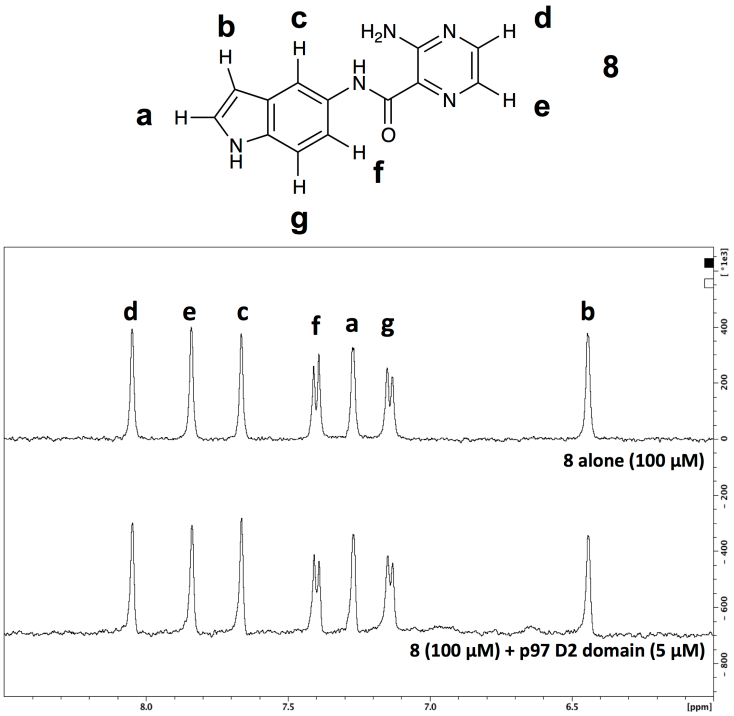


**Figure S3. Analysis of compound 8 by 1-D NMR in the presence of p97 D2 domain.** 100 µM compound **8** reference and the same concentration in the presence of 5 µM p97 D2.

**Table S1.** Inactive indole amide analogs (p97 ADP-Glo $IC_{50}$ > 50 µM)

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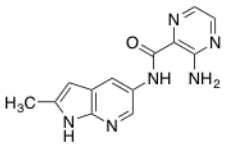
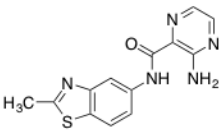
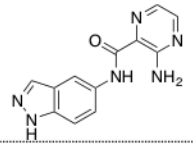
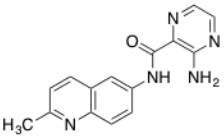
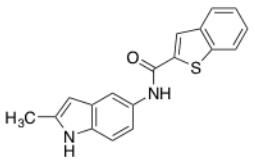
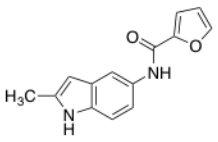
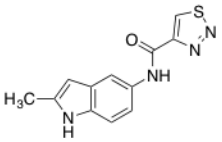
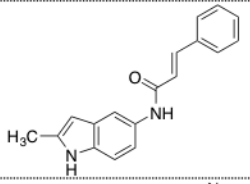
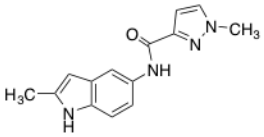
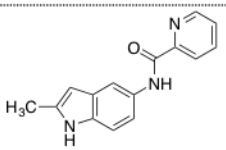
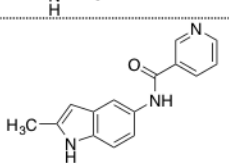
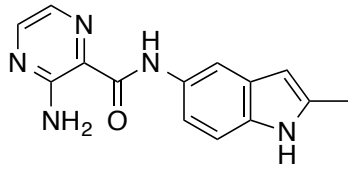
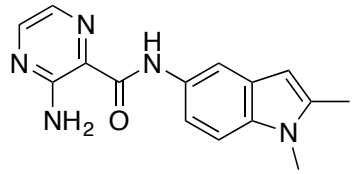
|  |  | **Inactive Analogs** |
|  |  |  |
|  |  |  |
|  |  |  |

**General:** All reactions were performed under an argon atmosphere and all glassware was flame dried prior to use. Reactions carried out at -78 $^{o}$C employed a dry ice/acetone bath. Anhydrous tetrahydrofuran and diethyl ether were distilled from sodium benzophenone ketyl; anhydrous dichloromethane and toluene were distilled from $CaH_2$; alternatively, the same solvents were obtained from a solvent purification system using alumina columns. All other solvents and reagents were used as obtained from commercial sources without further purification unless noted. Reactions were monitored by TLC analysis (EMD Millipore pre-coated silica gel 60 $F_{254}$ plates, 250 μm layer thickness) and visualization was accomplished with a 254 nm UV light or staining with a PMA solution (5 g of phosphomolybdic acid in 100 mL of 95% EtOH), *p*-anisaldehyde solution (2.5 mL of *p*-anisaldehyde, 2 mL of AcOH, and 3.5 mL of conc. $H_2SO_4$ in 100 mL of 95% EtOH), CAM solution (5 g of cerium sulfate, 25 g of ammonium molybdate, 50 mL of conc. $H_2SO_4$ and 450 mL of $H_2O$) or a $KMnO_4$ solution (1.5 g of $KMnO_4$ and 1.5 g of $K_2CO_3$ in 100 mL of a 0.1% NaOH solution). Purifications by chromatography were performed using $SiO_2$ (SiliaFlash® F60, Silicycle) or using an ISCO-Rf flash chromatography system. $^{1}H/^{13}C$ NMR spectra were recorded on a Bruker Avance 300/75 MHz, 400/100 MHz, or 500/125 MHz instruments. Chemical shifts were reported in parts per million with the residual solvent peak used as the internal standard ($^{1}H/^{13}C$: $CDCl_3$, 7.26/77.2 ppm; DMSO, 2.50/39.5 ppm, acetone, 2.05/29.8). Chemical shifts were tabulated as follows: chemical shift, multiplicity (s = singlet, d = doublet, t = triplet, q = quarter, sept = septet dd = doublet of doublet, dt = doublet of triplet, m = multiplet, b = broad, app = apparent), coupling constants, and integration. All 1D NMR spectra were processed using Mestrelalb iNMR. IR spectra were obtained on an Identity IR-ATR spectrometer. Microwave reactions were performed using a Biotage Initiator in glass microwave vials (cap sealed) with continuous magnetic stirring and an external surface temperature sensor. Melting points (uncorrected) were determined using a Mel-Temp instrument. HRMS data were obtained on a Thermo Scientific Exactive HRMS coupled to a Thermo Scientific Accela HPLC system using a 2.1 x 50 mm 3.5 μm Waters XTerra $C_{18}$ column eluting with MeCN/$H_2O$ containing 0.1% formic acid. Compound purity was assessed using the same HPLC system with either the PDA or an Agilent 385 ELSD. All final samples passed QC based on >95% purity by LC/MS/ELSD analysis.



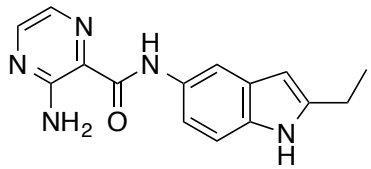
**3-Amino-*N*-(2-methyl-1*H*-indol-5-yl)pyrazine-2-carboxamide (SMDC818909).** **General procedure A:** To a solution of 3-aminopyrazine-2-carboxylic acid (0.139 g, 0.999 mmol), 2-methyl-1*H*-indol-5-amine (0.146 g, 0.999 mmol) in DMF (3 mL), was added TEA (0.56 mL, 4.0 mmol) and HATU (0.456 g, 1.20 mmol). The mixture was stirred at room temperature under Ar atmosphere for 12 h. The reaction mixture was diluted with EtOAc, and the organic phase was washed with $H_2O$ and brine, dried ($Na_2SO_4$), and concentrated. The residue was purified by chromatography on $SiO_2$ (hexanes/EtOAc, 50%) to give 3-amino-*N*-(2-methyl-1*H*-indol-5-yl)pyrazine-2-carboxamide (**SMDC818909**, 0.140 g, 52%) as a yellow solid: Mp 200-201 °C; IR (neat) 3247, 3327, 3141, 1646, 1610, 1539, 1431, 1198, 792 $cm^{-1}$; $^1H$ NMR (300 MHz, DMSO-$d_6$) δ 10.86 (s, 1 H), 10.23 (s, 1 H), 8.26 (d, $J$ = 2.3 Hz, 1 H), 7.91-7.89 (m, 2 H), 7.60 (bs, 2 H), 7.33 (dd, $J$ = 8.6, 1.9 Hz, 1 H), 7.22 (d, $J$ = 8.7 Hz, 1 H), 6.10 (s, 1 H), 2.37 (s, 3 H); $^{13}C$ NMR (125 MHz, DMSO-$d_6$) δ 163.8, 155.3, 146.8, 136.4, 133.3, 130.8, 129.7, 128.4, 126.0, 114.5, 111.0, 110.1, 99.3, 13.4; HRMS ($ESI^+$) *m/z* calcd for $C_{14}H_{14}N_5O$ $[M+H]^+$ 268.1193, found 268.1195; ELS purity (100%).



**3-Amino-*N*-(1,2-dimethyl-1*H*-indol-5-yl)pyrazine-2-carboxamide (6). General Procedure B:** To a solution of 3-aminopyrazine-2-carboxylic acid (0.100 g, 0.719 mmol) in DMF (2.2 mL), TEA (0.40 mL, 2.9 mmol) and HATU (0.328 g, 0.863 mmol) were added. The mixture was stirred at room temperature for 40 min followed by the addition of 1,2-dimethyl-1*H*-indol-5-amine[2] (0.115 g, 0.719 mmol). The reaction mixture was stirred for 12 h at room temperature under inert atmosphere. The resulting mixture was

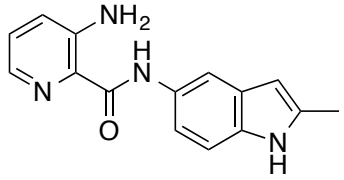
[2] Synthesis of **1,2-Dimethyl-5-nitro-1*H*-indole** according to Krichevskii, É.S.; Alekseeva, L.M.; Granik, V.G. *Chem. Heterocycl. Compd.* **1990**, *26*, 1235-1238; synthesis of **1,2-Dimethyl-1*H*-indol-5-amine** according to Kuyper, L.F.; Baccanari, D.P.; Jones, M.L.; Hunter, R.N.; Tansik, R.L.; Joyner, S.S.; Boytos, C.M.; Rudolph, S.K.; Knick, V.; Wilson, H.R.; Caddell, J.M.; Friedman, H.S.; Comley, J.C.W.; Stables, J.N. *J. Med. Chem.* **1996**, *39*, 892-903.

diluted with EtOAc, and the organic phase was washed with $H_2O$ and brine, dried ($Na_2SO_4$), and concentrated. The residue was adsorbed onto $SiO_2$ and purified by chromatography (EtOAc/hexanes, 60%) to give 3-amino-*N*-(1,2-dimethyl-1*H*-indol-5-yl)pyrazine-2-carboxamide (**6**, 0.163 g, 81%) as a deep orange solid: Mp 183-185 °C; IR (neat) 3386, 3334, 3248, 3140, 1658, 1606, 1534, 1465, 1430, 1198, 1087, 846, 654 $cm^{-1}$; $^1H$ NMR (400 MHz, DMSO-$d_6$) δ 10.29 (s, 1 H), 8.26 (d, $J$ = 2.3 Hz, 1 H), 7.94 (d, $J$ = 1.7 Hz, 1 H), 7.91 (d, $J$ = 2.3 Hz, 1 H), 7.61 (bs, 2 H), 7.41 (dd, $J$ = 8.8, 1.9 Hz, 1 H), 7.33 (d, $J$ = 8.7 Hz, 1 H), 6.19 (s, 1 H), 3.65 (s, 3 H), 2.39 (s, 3 H); $^{13}C$ NMR (100 MHz, DMSO-$d_6$) δ 163.9, 155.4, 146.9, 137.8, 134.3, 130.9, 130.1, 127.2, 126.0, 114.5, 111.0, 108.9, 99.2, 29.3, 12.4; HRMS ($ESI^+$) *m/z* calcd for $C_{15}H_{16}N_5O$ $[M+H]^+$ 282.1349, found 282.1347; ELS purity (100%).

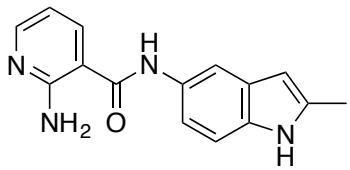


**3-Amino-*N*-(2-ethyl-1*H*-indol-5-yl)pyrazine-2-carboxamide (7). General Procedure C:** A solution of 3-aminopyrazine-2-carboxylic acid (0.057 g, 0.40 mmol) in DMF (2.0 mL) was treated with HATU (0.16 g, 0.41 mmol) followed by DIPEA (0.10 mL, 0.60 mmol) at room temperature. After 15 min, 2-ethyl-1*H*-indol-5-amine (0.063 g, 0.39 mmol) in DMF (2.0 mL) was added dropwise. After 17 h, the solution was extracted with EtOAc, and the organic phase was washed with sat. $NaHCO_3$ and brine, dried ($Na_2SO_4$), and concentrated. The residue was adsorbed onto $SiO_2$ and purified by chromatography on $SiO_2$ (ISCO-Rf, 15 min gradient; EtOAc/hexanes, 0-100%) to give semi-pure solid that was suspended in $Et_2O$/hexanes (2:1) and filtered to give 3-amino-*N*-(2-ethyl-1*H*-indol-5-yl)pyrazine-2-carboxamide (**7**, 0.059 g, 54%) as a yellow-brown solid: Mp 223-226 °C; IR (neat) 3295, 3057, 2974, 1649, 1612, 1543, 1450, 1316, 1193, 807 $cm^{-1}$; $^1H$ NMR (400 MHz, DMSO-$d_6$) δ 10.87 (s, 1 H), 10.24 (s, 1 H), 8.26 (d, $J$ = 2.3 Hz, 1 H), 7.91 (d, $J$ = 2.3 Hz, 1 H), 7.90 (d, $J$ = 2.0 Hz, 1 H), 7.60 (bs, 2 H), 7.34 (dd, $J$ = 8.7, 2.0 Hz, 1 H), 7.22 (d, $J$ = 8.6 Hz, 1 H), 6.12 (app dd, $J$ = 2.0, 0.9 Hz, 1 H), 2.72 (q, $J$ = 7.6 Hz, 2 H), 1.27 (t, $J$ = 7.6 Hz, 3 H); $^{13}C$ NMR (100 MHz, DMSO-$d_6$) δ 163.8, 155.4, 146.9, 142.7, 133.2, 130.8, 129.7, 128.1, 126.0, 114.6, 111.1, 110.3, 97.7, 20.9, 13.4;

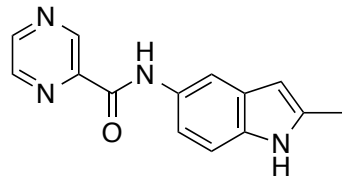
HRMS (ESI$^+$) *m/z* calcd for $C_{15}H_{16}N_5O$ $[M+H]^+$ 282.1349, found 282.1348; ELS purity (100%).



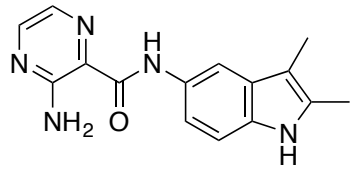
**3-Amino-*N*-(2-methyl-1*H*-indol-5-yl)picolinamide (2).** Prepared according to general procedure A from 2-methyl-1*H*-indol-5-amine and 3-aminopicolinic acid. 3-Amino-*N*-(2-methyl-1*H*-indol-5-yl)picolinamide (**2**, 0.149 g, 56%) was obtained as a pale yellow solid: Mp 159-160 °C; IR (neat) 3465, 3348, 3321, 3277, 1644, 1590, 1521, 1478, 1215, 781 cm$^{-1}$; $^1$H NMR (400 MHz, DMSO-$d_6$) δ 10.84 (s, 1 H), 10.18 (s, 1 H), 7.90 (d, $J$ = 1.9 Hz, 1 H), 7.88 (dd, $J$ = 4.2, 1.5 Hz, 1 H), 7.31-7.28 (m, 2 H), 7.23-7.19 (m, 2 H), 6.92 (bs, 2 H), 6.09 (app t, $J$ = 1.0 Hz, 1 H), 2.37 (d, $J$ = 0.6 Hz, 3 H); $^{13}$C NMR (100 MHz, DMSO-$d_6$) δ 165.2, 146.6, 136.3, 135.5, 133.1, 130.0, 128.7, 128.5, 127.4, 124.8, 114.1, 110.3, 110.2, 99.2, 13.4; HRMS (ESI$^+$) *m/z* calcd for $C_{15}H_{15}N_4O$ $[M+H]^+$ 267.1240, found 267.1239; ELS purity (100%).



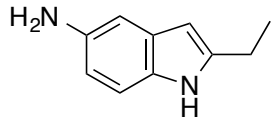
**2-Amino-*N*-(2-methyl-1*H*-indol-5-yl)nicotinamide (3).** Prepared according to general procedure B from 2-methyl-1*H*-indol-5-amine and 2-aminonicotinic acid. 2-Amino-*N*-(2-methyl-1*H*-indol-5-yl)nicotinamide (**3**, 0.164 g, 85%) was obtained as a brown solid: Mp 221-222 °C; IR (neat) 3422, 3381, 3320, 3146, 1636, 1623, 1571, 1476, 1249, 805, 782, 772 cm$^{-1}$; $^1$H NMR (300 MHz, DMSO-$d_6$) δ 10.84 (s, 1 H), 9.95 (s, 1 H), 8.10 (dd, $J$ = 4.7, 1.3 Hz, 1 H), 8.04 (dd, $J$ = 7.6, 1.2 Hz, 1 H), 7.74 (s, 1 H), 7.21 (s, 2 H), 6.95 (s, 2 H), 6.65 (dd, $J$ = 7.6, 4.8 Hz, 1 H), 6.09 (s, 1 H), 2.37 (s, 3 H); $^{13}$C NMR (100 MHz, DMSO-$d_6$) δ 166.1, 158.7, 150.9, 136.9, 136.2, 133.2, 130.3, 128.3, 115.2, 111.8, 111.4, 110.8, 110.0, 99.2, 13.4; HRMS (ESI$^+$) *m/z* calcd for $C_{15}H_{15}N_4O$ $[M+H]^+$ 267.1240, found 267.1238; ELS purity (100%).



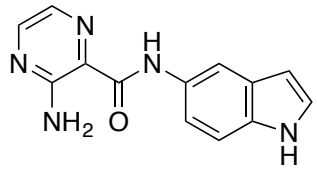
***N*-(2-Methyl-1*H*-indol-5-yl)pyrazine-2-carboxamide (4).** Prepared according to general procedure A from 2-methyl-1*H*-indol-5-amine and pyrazine-2-carboxylic acid. *N*-(2-Methyl-1*H*-indol-5-yl)pyrazine-2-carboxamide (**4**, 0.081 g, 32%) was obtained as an orange solid: Mp 208-209 °C; IR (neat) 3346, 3135, 2926, 1679, 1541, 1487, 1021, 781 $cm^{-1}$; $^{1}H$ NMR (500 MHz, DMSO-$d_6$) δ 10.89 (s, 1 H), 10.43 (s, 1 H), 9.29 (d, $J$ = 1.4 Hz, 1 H), 8.91 (d, $J$ = 2.5 Hz, 1 H), 8.80-8.79 (m, 1H), 7.96 (d, $J$ = 1.8 Hz, 1 H), 7.42 (dd, $J$ = 8.6, 2.0 Hz, 1 H), 7.24 (d, $J$ = 8.6 Hz, 1 H), 6.11 (app t, $J$ = 0.9 Hz, 1 H), 2.37 (s, 3 H); $^{13}C$ NMR (125 MHz, DMSO-$d_6$) δ 160.9, 147.3, 145.5, 143.8, 143.1, 136.4, 133.4, 129.8, 128.4, 114.5, 111.1, 110.2, 99.3, 13.4; HRMS ($ESI^+$) *m/z* calcd for $C_{14}H_{13}N_4O$ $[M+H]^+$ 253.1084, found 253.1083; ELS purity (100%).



**3-Amino-*N*-(2,3-dimethyl-1*H*-indol-5-yl)pyrazine-2-carboxamide (5).** Prepared according to general procedure A from 2,3-dimethyl-1*H*-indol-5-amine and 3-aminopyrazine-2-carboxylic acid. 3-Amino-*N*-(2,3-dimethyl-1*H*-indol-5-yl)pyrazine-2-carboxamide (**5**, 0.070 g, 50%) was obtained as a yellow solid: Mp 249-250 °C; IR (neat) 3394, 3336, 3128, 1659, 1608, 1534, 1437, 814 $cm^{-1}$; $^{1}H$ NMR (400 MHz, DMSO-$d_6$) δ 10.62 (s, 1 H), 10.23 (s, 1 H), 8.26 (d, $J$ = 2.3 Hz, 1 H), 7.90 (d, $J$ = 2.3 Hz, 1 H), 7.87 (d, $J$ = 1.9 Hz, 1 H), 7.60 (bs, 2 H), 7.32 (dd, $J$ = 8.6, 2.0 Hz, 1 H), 7.17 (dd, $J$ = 8.5, 0.4 Hz, 1 H), 2.30 (s, 3 H), 2.14 (d, $J$ = 0.5 Hz, 3 H); $^{13}C$ NMR (125 MHz, DMSO-$d_6$) δ 163.7, 155.3, 146.8, 132.35, 132.24, 130.8, 129.3, 128.7, 126.0, 114.4, 110.0, 109.4, 105.2, 11.2, 8.4; HRMS ($ESI^+$) *m/z* calcd for $C_{15}H_{16}N_5O$ $[M+H]^+$ 282.1349, found 282.1343; ELS purity (100%).

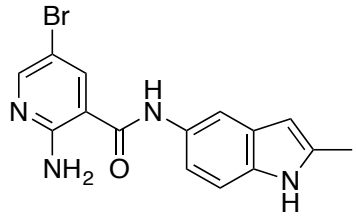


**2-Ethyl-1*H*-indol-5-amine.** A solution of 2-ethyl-5-nitro-1*H*-indole[3] (0.15 g, 0.78 mmol) in EtOH (4.0 mL) was evacuated with Ar (2x) and treated with 10% Pd/C (0.039 g). The solution was evacuated with Ar (2x) and subjected to $H_2$ (~1 atm, balloon). After 4 h, the solution was filtered through Celite, rinsed with MeOH, and concentrated. The residue was slurried with $CH_2Cl_2$ and concentrated (2x) to give 2-ethyl-1*H*-indol-5-amine (0.118 g, 94%) as a light brown solid that was used without further purification: $^1$H NMR (400 MHz, $CDCl_3$) δ 7.68 (bs, 1 H), 7.09 (d, *J* = 8.4 Hz, 1 H), 6.85 (d, *J* = 2.1 Hz, 1 H), 6.58 (dd, *J* = 8.4, 2.2 Hz, 1 H), 6.08 (app d, *J* = 1.0 Hz, 1 H), 3.46 (bs, 2 H), 2.74 (q, *J* = 7.5 Hz, 2 H), 1.32 (t, *J* = 7.6 Hz, 3 H).

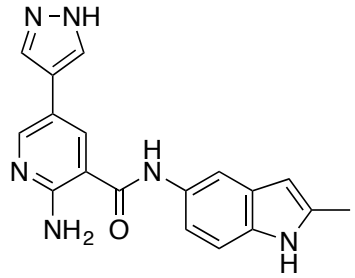


**3-Amino-*N*-(1*H*-indol-5-yl)pyrazine-2-carboxamide (8).** Prepared according to general procedure C from 1*H*-indol-5-amine and 3-aminopyrazine-2-carboxylic acid. 3-Amino-*N*-(1*H*-indol-5-yl)pyrazine-2-carboxamide (**8**, 0.059 g, 58%) was obtained as a light yellow solid: Mp 187-189 °C; IR (neat) 3422, 3327, 3059, 1649, 1530, 1450, 1420, 1340, 1215, 1192, 1077, 982, 727 cm$^{-1}$; $^1$H NMR (400 MHz, DMSO-*d*$_6$) δ 11.05 (bs, 1 H), 10.30 (d, *J* = 2.7 Hz, 1 H), 8.28-8.26 (m, 1 H), 8.03 (app t, *J* = 2.0 Hz, 1 H), 7.93-7.91 (m, 1 H), 7.61 (bs, 2 H), 7.45-7.41 (m, 1 H), 7.36-7.32 (m, 2 H), 6.40 (app t, *J* = 2.3 Hz, 1 H); $^{13}$C NMR (100 MHz, DMSO-*d*$_6$) δ 164.0, 155.4, 146.9, 133.1, 130.9, 129.9, 127.4, 126.02, 125.96, 115.8, 111.9, 111.1, 101.2; HRMS (ESI$^+$) *m/z* calcd for $C_{13}H_{12}N_5O$ $[M+H]^+$ 254.1036, found 254.1037; ELS purity (100%).

[3] Jiao, L.; Bach, T. *J. Am. Chem. Soc*. **2011**, *133*, 12990-12993.

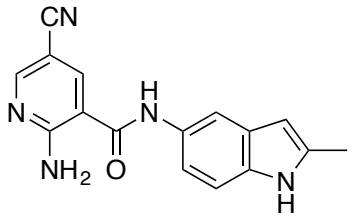


**2-Amino-5-bromo-*N*-(2-methyl-1*H*-indol-5-yl)nicotinamide.** Prepared according to general procedure B from 2-methyl-1*H*-indol-5-amine and 2-amino-5-bromonicotinic acid. 2-Amino-5-bromo-*N*-(2-methyl-1*H*-indol-5-yl)nicotinamide (0.319 g, 80%) was obtained as a pink solid: Mp 235-237 °C; IR (neat) 3362, 3228, 1610, 1565, 1548, 1522, 1478, 1446, 1297, 1241, 803, 798, 766 $cm^{-1}$; $^1H$ NMR (400 MHz, DMSO-$d_6$) δ 10.87 (s, 1 H), 10.07 (s, 1 H), 8.21 (dd, $J$ = 21.5, 2.1 Hz, 2 H), 7.74 (s, 1 H), 7.22 (s, 2 H), 7.16 (s, 2 H), 6.10 (s, 1 H), 2.37 (s, 3 H); $^{13}C$ NMR (100 MHz, DMSO-$d_6$) δ 164.8, 157.5, 151.2, 138.6, 136.3, 133.3, 130.1, 128.4, 115.1, 112.1, 111.8, 110.0, 104.1, 99.2, 13.4; HRMS ($ESI^+$) *m/z* calcd for $C_{15}H_{14}N_4OBr$ $[M+H]^+$ 345.0346, found 345.0342; ELS purity (100%).

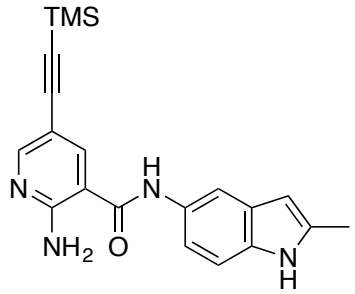


**2-Amino-*N*-(2-methyl-1*H*-indol-5-yl)-5-(1*H*-pyrazol-4-yl)nicotinamide (9).** To a solution of 2-amino-5-bromo-*N*-(2-methyl-1*H*-indol-5-yl)nicotinamide (0.12 g, 0.35 mmol) in deoxygenated DMF (3.3 mL) was added $Pd(PPh_3)_2Cl_2$ (0.01 g, 0.01 mmol) and pyrazole-4-boronic acid pinacol ester (0.10 g, 0.52 mmol) and after 10 min $K_3PO_4$ (0.15 g, 0.70 mmol) and deoxygenated water (0.8 mL). The reaction was subjected to microwave irradiation at 130 °C for 3 h. The resulting mixture was diluted with EtOAc, and the organic phase was washed with sat. LiCl, dried ($MgSO_4$), and concentrated. The residue was purified by chromatography on $SiO_2$ (ISCO-Rf, EtOAc/hexanes, 0-100%). 2-Amino-*N*-(2-methyl-1*H*-indol-5-yl)-5-(1*H*-pyrazol-4-yl)nicotinamide (**9**, 0.03 g, 23%) was obtained as a yellow solid: Mp 180 °C; IR (neat) 3635, 3369, 2971, 1638, 1584,

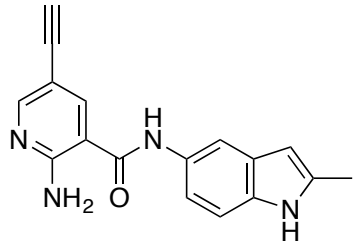
1531, 1478, 1450, 1244, 835, 792 $cm^{-1}$; $^1H$ NMR (400 MHz, acetone-$d_6$) δ 9.99 (bs, 1 H), 9.47 (s, 1 H), 8.43 (d, *J* = 1.5 Hz, 1 H), 8.30 (d, *J* = 1.9 Hz, 1 H), 7.99 (s, 2 H), 7.88 (d, *J* = 1.8 Hz, 1 H), 7.32 (dd, *J* = 8.6, 2.0 Hz, 1 H), 7.27 (d, *J* = 8.6 Hz, 1 H), 6.78 (bs, 2 H), 6.15 (dt, *J* = 1.9, 0.9 Hz, 1 H), 2.42 (s, 3 H); $^{13}C$ NMR (100 MHz, acetone-$d_6$) δ 167.1, 158.5, 149.0, 137.2, 134.8, 134.4, 131.7, 131.0 (b), 130.0, 119.7, 118.8, 116.1, 112.8, 112.0, 110.9, 100.6, 13.6; HRMS ($ESI^+$) *m/z* calcd for $C_{18}H_{17}N_6O$ $[M+H]^+$ 333.1458, found 333.1456; ELS purity (100%).




**2-Amino-5-cyano-*N*-(2-methyl-1*H*-indol-5-yl)nicotinamide (10).** To a solution of 2-amino-5-bromo-*N*-(2-methyl-1*H*-indol-5-yl)nicotinamide (0.12 g, 0.35 mmol) in deoxygenated DMF (1.8 mL) was added CuCN (0.047 g, 0.52 mmol), TEA (0.15 mL, 1.0 mmol), and Pd(dppf)$Cl_2$ (0.03 g, 0.03 mmol). The reaction was heated at 130 °C for 12 h and then cooled to room temperature. The mixture was extracted with EtOAc, and the organic phase was washed with $H_2O$, dried ($MgSO_4$), and concentrated. The residue was purified by chromatography on $SiO_2$ (ISCO-Rf, EtOAc/hexanes, 50-100%). 2-Amino-5-cyano-*N*-(2-methyl-1*H*-indol-5-yl)nicotinamide (**10**, 0.02 g, 15%) was obtained as a pale yellow solid: Mp 220 °C; IR (neat) 3364, 3141, 2225, 1644, 1627, 1515, 1411, 1281, 964, 807 $cm^{-1}$; $^1H$ NMR (400 MHz, acetone-$d_6$) δ 10.01 (bs, 1 H), 9.49 (s, 1 H), 8.44 (d, *J* = 1.7 Hz, 1 H), 8.41 (d, *J* = 1.4 Hz, 1 H), 7.89 (s, 1 H), 7.63 (bs, 2 H), 7.31 (dd, *J* = 8.6, 1.6 Hz, 1 H), 7.27 (d, *J* = 8.6 Hz, 1 H), 6.16 (s, 1 H), 2.42 (s, 3 H); $^{13}C$ NMR (125 MHz, acetone-$d_6$) δ 165.4, 161.5, 155.7, 140.3, 137.4, 135.0, 131.4, 130.1, 118.5, 115.9, 112.7, 111.6, 111.0, 100.7, 96.8, 13.6; HRMS ($ESI^+$) *m/z* calcd for $C_{16}H_{13}N_5O$ $[M+H]^+$ 292.1193, found 292.1190; ELS purity (100%).

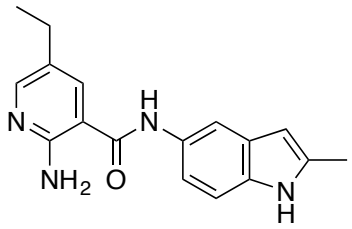



**2-Amino-*N*-(2-methyl-1*H*-indol-5-yl)-5-((trimethylsilyl)ethynyl)nicotinamide**. To a solution of 2-amino-5-bromo-*N*-(2-methyl-1*H*-indol-5-yl)nicotinamide (0.15 g, 0.43 mmol) in deoxygenated DMF (2.7 mL) was added TMS acetylene (0.15 mL, 1.0 mmol), $Pd(PPh_3)_2Cl_2$ (0.06 g, 0.09 mmol), and CuI (0.025 g, 0.13 mmol) under argon atmosphere. The reaction was subjected to microwave irradiation at 130 °C for 40 min. The resulting mixture was diluted with EtOAc, and the organic phase was washed with water and sat. LiCl, dried ($MgSO_4$), and concentrated. The residue was purified by chromatography on $SiO_2$ (ISCO-Rf, EtOAc/hexanes, 10-50%) to give 2-amino-*N*-(2-methyl-1*H*-indol-5-yl)-5-((trimethylsilyl)ethynyl)nicotinamide (0.11 g, 41%, approx. 70% pure) as an off-white foam: $^1$H NMR (300 MHz, $CDCl_3$) δ 8.31 (d, *J* = 2.0 Hz, 1 H), 7.91 (bs, 1 H), 7.86 (d, *J* = 1.9 Hz, 1 H), 7.70-7.68 (m, 2 H), 7.26 (d, *J* = 8.5 Hz, 2 H), 7.16 (dd, *J* = 8.6, 2.0 Hz, 1 H), 6.56 (bs, 1 H), 6.21 (app t, *J* = 1.0 Hz, 1 H), 2.45 (s, 3 H), 0.26 (s, 9 H); HRMS ($ESI^+$) *m/z* calcd for $C_{20}H_{23}N_4OSi$ $[M+H]^+$ 363.1636, found 363.1637.



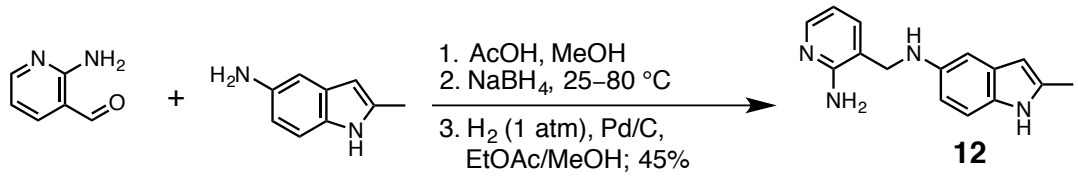

**2-Amino-5-ethynyl-*N*-(2-methyl-1*H*-indol-5-yl)nicotinamide.** To a solution of 2-amino-*N*-(2-methyl-1*H*-indol-5-yl)-5-((trimethylsilyl)ethynyl)nicotinamide (0.10 g, 0.19 mmol) in MeOH (1.7 mL) was added $K_2CO_3$ (0.081 g, 0.58 mmol) and the reaction was stirred at room temperature for 3 h. The reaction mixture was diluted with EtOAc, washed with sat. $NaHCO_3$, water, and brine. The organic layer was dried ($MgSO_4$) and concentrated. The mixture was purified by chromatography on $SiO_2$ (ISCO-Rf,

EtOAc/hexanes, 0-50%). 2-Amino-5-ethynyl-*N*-(2-methyl-1*H*-indol-5-yl)nicotinamide (0.25 g, 45%) was obtained as an off-white solid: HRMS ($ESI^+$) *m/z* calcd for $C_{17}H_{15}N_4O$ $[M+H]^+$ 291.1240, found 291.1238.



**2-Amino-5-ethyl-*N*-(2-methyl-1*H*-indol-5-yl)nicotinamide (11).** To a solution of 2-amino-5-ethynyl-*N*-(2-methyl-1*H*-indol-5-yl)nicotinamide (0.025 g, 0.086 mmol) in deoxygenated EtOH (1.7 mL) was added 20% Pd/C (0.002 g). $H_2$ gas (~1 atm, balloon) was bubbled into the reaction, and the reaction was kept under an atmosphere of $H_2$ for 12 h. The reaction was filtered through Celite, washed with EtOH, and concentrated. The mixture was purified by chromatography on $SiO_2$ (EtOAc/$CH_2Cl_2$, 0-50%). 2-Amino-5-ethyl-*N*-(2-methyl-1*H*-indol-5-yl)nicotinamide (**11**, 0.019 g, 75%) was obtained as a pale yellow solid: Mp 115-116 °C; IR (neat) 3480, 3399, 3285, 3148, 2963, 2870, 1628, 1561, 1548, 1451, 1322, 1217, 1082, 865, 798, 766 $cm^{-1}$; $^1H$ NMR (300 MHz, acetone-$d_6$) δ 9.96 (bs, 1 H), 9.31 (s, 1 H), 8.00 (d, *J* = 2.2 Hz, 1 H), 7.95 (d, *J* = 2.2 Hz, 1 H), 7.86 (d, *J* = 1.6 Hz, 1 H), 7.30 (dd, *J* = 8.6, 1.9 Hz, 1 H), 7.24 (d, *J* = 8.6 Hz, 1 H), 6.59 (bs, 2 H), 6.14 (app t, *J* = 0.9 Hz, 1 H), 2.53 (q, *J* = 7.6 Hz, 2 H), 2.42 (s, 3 H), 1.20 (t, *J* = 7.6 Hz, 3 H); $^{13}C$ NMR (125 MHz, acetone-$d_6$) δ 167.2, 158.6, 151.4, 137.2, 136.5, 134.8, 131.8, 130.1, 128.0, 116.1, 112.7, 111.7, 110.9, 100.6, 25.7, 16.2, 13.6; HRMS ($ESI^+$) *m/z* calcd for $C_{17}H_{19}N_4O$ $[M+H]^+$ 295.1553, found 295.1551; ELS purity (99.7%).

**Scheme S1.** Synthesis of reduced amide analog **12**

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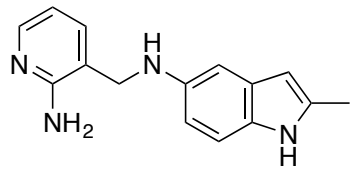
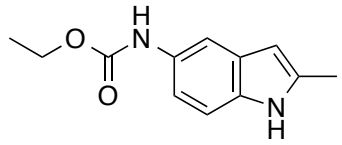
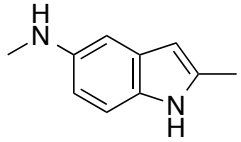


***N*-((2-Aminopyridin-3-yl)methyl)-2-methyl-1*H*-indol-5-amine (12).** A mixture of 2-methyl-1*H*-indol-5-amine (0.053 g, 0.36 mmol) and 2-aminonicotinaldehyde (0.044 g, 0.36 mmol) in MeOH (3.0 mL) was treated with AcOH (0.050 mL, 0.87 mmol) at room temperature. After 17 h, the heterogeneous brown solution was treated with $NaBH_4$ (0.039 g, 1.0 mmol). LCMS indicated a small amount of product formation after 5 min. After 3 h, additional $NaBH_4$ (0.042 g, 1.1 mmol) was added, and the solution was stirred at room temperature for 12 h. The heterogeneous reaction mixture was subsequently heated at 80 °C for an additional 12 h. The brown solution was cooled to room temperature and extracted with EtOAc. The organic phase was washed with sat. $NaHCO_3$ and brine, dried ($Na_2SO_4$), and concentrated. The brown residue was adsorbed onto $SiO_2$ and purified by chromatography on $SiO_2$ (ISCO-Rf, 15 min gradient; MeOH/$CH_2Cl_2$, 0-10%) to give the intermediate imine as a yellow solid (0.020 g, 22%) and **12** as a light brown solid (0.028 g, 31%). The imine (0.020 g, 0.08 mmol) in MeOH/EtOAc (3 mL, 1:1) was evacuated, flushed with Ar (2x), treated with 10% Pd/C (0.009 g), and subjected to $H_2$ (~1 atm, balloon) for 4 h. The solution was filtered through Celite, rinsed with MeOH and EtOAc, and concentrated. The residue was adsorbed onto $SiO_2$ and purified by chromatography on $SiO_2$ (ISCO-Rf, 15 min gradient; MeOH/$CH_2Cl_2$, 0-5%) to give additional product **12** (0.013 g, 14%). *N*-((2-aminopyridin-3-yl)methyl)-2-methyl-1*H*-indol-5-amine was obtained in a combined yield (**12**, 0.041 g, 45%) as a brown solid: Mp 139-141 °C; IR (neat) 3465, 3385, 3126, 2975, 2934, 1629, 1567, 1450, 1279, 1193, 1077, 797, 768 $cm^{-1}$; $^1H$ NMR $^1H$ NMR (500 MHz; DMSO-$d_6$) δ 10.40 (s, 1 H), 7.81 (dd, $J$ = 4.8, 1.5 Hz, 1 H), 7.38 (dd, $J$ = 7.2, 1.2 Hz, 1 H), 6.99 (d, $J$ = 8.3 Hz, 1 H), 6.50-6.45 (m, 3 H), 5.83 (app t, $J$

= 0.8 Hz, 1 H), 5.77 (s, 2 H), 5.40 (t, *J* = 5.6 Hz, 1 H), 4.02 (d, *J* = 5.5 Hz, 2 H), 2.28 (s, 3 H); $^{13}$C NMR (125 MHz, $CDCl_3$) δ 158.2, 147.4, 141.9, 137.5, 135.9, 131.0, 130.1, 118.2, 114.1, 111.3, 111.0, 103.4, 100.1, 48.5, 13.9; HRMS ($ESI^+$) *m/z* calcd for $C_{15}H_{17}N_4$ $[M+H]^+$ 253.1448, found 253.1449; ELS purity (100%).

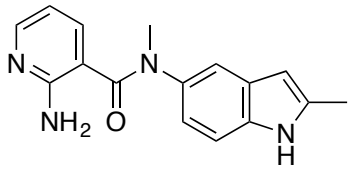


**Ethyl (2-methyl-1*H*-indol-5-yl)carbamate.** A solution of 2-methyl-1*H*-indol-5-amine (0.230 g, 1.57 mmol) in THF (3.0 mL) was treated with TEA (0.182 g, 1.80 mmol, 0.25 mL) at 0 °C followed by dropwise addition of ethyl chloroformate (0.182 g, 1.67 mmol, 0.16 mL). After 10 min, the solution was warmed to room temperature and stirred for 2 h. The solution was then extracted with EtOAc, and the organic layer was washed with brine, dried ($Na_2SO_4$), and concentrated. The residue was adsorbed onto $SiO_2$ and purified by chromatography on $SiO_2$ (ISCO-Rf, 15 min gradient; EtOAc/hexanes, 0-100%) to give an off-white solid. The solid was dissolved in $CH_2Cl_2$ and washed with 1M HCl and brine. The organic phase was dried ($Na_2SO_4$) and concentrated to give ethyl (2-methyl-1*H*-indol-5-yl)carbamate (0.24 g, 69%) as an off-white solid: $^1$H NMR (300 MHz, $CDCl_3$) δ 8.03 (bs, 1 H), 7.53 (bs, 1 H), 7.11-7.02 (m, 2 H), 6.66 (bs, 1 H), 6.14 (app s, 1 H), 4.25 (q, *J* = 7.1 Hz, 2 H), 2.36 (s, 3 H), 1.32 (t, *J* = 7.1 Hz, 3 H).

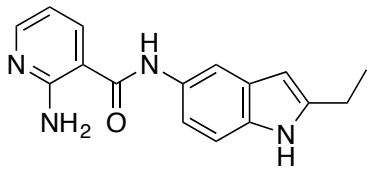


***N*,2-Dimethyl-1*H*-indol-5-amine**. To a solution of ethyl (2-methyl-1*H*-indol-5-yl)carbamate (0.236 g, 1.08 mmol) in dry THF (3.0 mL) was added LAH (3.0 mL, 1M in $Et_2O$) at 0 °C. After complete addition, the reaction mixture was heated to reflux. After 2 h, the solution was cooled to room temperature and quenched by the slow addition of sat. $Na_2SO_4$. The mixture was extracted with EtOAc, washed with brine, dried ($Na_2SO_4$), and concentrated to give a dark brown solid residue. The residue was suspended in EtOAc, passed through a small plug of $SiO_2$, and concentrated to give *N*,2-dimethyl-1*H*-indol-5-amine (0.173 g, 100%) as a light brown solid that was used without further purification:

$^1$H NMR (400 MHz, $CDCl_3$) δ 7.67 (bs, 1 H), 7.08 (d, *J* = 8.5 Hz, 1 H), 6.78 (d, *J* = 2.2 Hz, 1 H), 6.56 (dd, *J* = 8.5, 2.3 Hz, 1 H), 6.10 (app t, *J* = 1.0 Hz, 1 H), 3.28 (bs, 1 H), 2.89 (s, 3 H), 2.38 (s, 3 H).

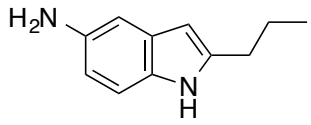


**2-Amino-*N*-methyl-*N*-(2-methyl-1*H*-indol-5-yl)nicotinamide (13).** Prepared according to general procedure B from *N*,2-dimethyl-1*H*-indol-5-amine and 2-aminonicotinic acid. 2-Amino-*N*-methyl-*N*-(2-methyl-1*H*-indol-5-yl)nicotinamide (**13,** 0.105 g, 86%) was obtained as a white solid: Mp 147-151 °C; IR (neat) 3301, 1612, 1571, 1450, 1377, 766 cm$^{-1}$; $^1$H NMR (400 MHz, DMSO-$d_6$) δ 10.95 (s, 1 H), 7.71 (dd, *J* = 4.7, 1.5 Hz, 1 H), 7.22 (d, *J* = 1.2 Hz, 1 H), 7.11 (d, *J* = 8.5 Hz, 1 H), 7.05 (dd, *J* = 7.5, 0.8 Hz, 1 H), 6.82 (dd, *J* = 8.5, 1.9 Hz, 1 H), 6.18 (dd, *J* = 7.3, 5.0 Hz, 1 H), 6.04-6.03 (m, 3 H), 3.34 (s, 3 H), 2.32 (s, 3 H); $^{13}$C NMR (100 MHz, DMSO-$d_6$) δ 168.8, 156.7, 148.3, 136.9, 136.5, 135.9, 134.5, 128.6, 119.0, 116.8, 114.9, 110.9, 110.7, 99.4, 38.2, 13.3; HRMS (ESI$^+$) *m/z* calcd for $C_{16}H_{17}N_4O$ $[M+H]^+$ 281.1397, found 281.1392; ELS purity (99.8%).

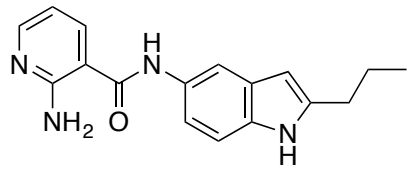


**2-Amino-*N*-(2-ethyl-1*H*-indol-5-yl)nicotinamide (14).** Prepared according to general procedure B from 2-ethyl-1*H*-indol-5-amine and 2-aminonicotinic acid. 2-Amino-*N*-(2-ethyl-1*H*-indol-5-yl)nicotinamide (**14,** 0.084 g, 83%) was obtained as a beige solid: Mp 177-180 °C; IR (neat) 3381, 3276, 3148, 1634, 1618, 1571, 1478, 1440, 1245, 839, 795, 767 cm$^{-1}$; $^1$H NMR (500 MHz, DMSO-$d_6$) δ 10.85 (s, 1 H), 9.95 (s, 1 H), 8.10 (dd, *J* = 4.7, 1.7 Hz, 1 H), 8.04 (dd, *J* = 7.7, 1.6 Hz, 1 H), 7.75 (s, 1 H), 7.25-7.21 (m, 2 H), 6.96 (s, 2 H), 6.65 (dd, *J* = 7.6, 4.8 Hz, 1 H), 6.12 (d, *J* = 1.1 Hz, 1 H), 2.73 (q, *J* = 7.5 Hz, 2 H), 1.28 (t, *J* = 7.6 Hz, 3 H); $^{13}$C NMR (125 MHz, DMSO-$d_6$) δ 166.1, 158.8, 151.1, 142.5, 136.8, 133.2, 130.3, 128.1, 115.3, 112.0, 111.4, 110.7, 110.1, 97.6, 20.9, 13.4;

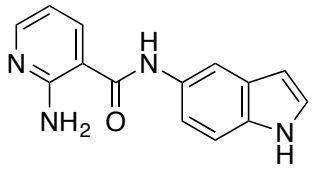
HRMS (ESI$^+$) *m/z* calcd for $C_{16}H_{17}N_4O$ $[M+H]^+$ 281.1397, found 281.1395; ELS purity (99.3%).



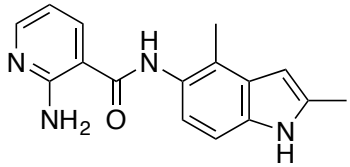
**2-Propyl-1*H*-indol-5-amine.** A solution of 5-nitro-2-propyl-1*H*-indole[3] (0.163 g, 0.798 mmol) in EtOH (4.0 mL) was evacuated with Ar (2x) and treated with 10% Pd/C (0.041 g). The solution was evacuated with Ar (2x) and subsequently subjected to $H_2$ atmosphere (~1 atm, balloon). After 4 h, the solution was filtered through Celite, eluted with MeOH, and concentrated. The residue concentrated with $CH_2Cl_2$ (2x) to give 2-propyl-1*H*-indol-5-amine (0.128 g, 92%) as a light brown solid that was used without further purification: $^1$H NMR (400 MHz, $CDCl_3$) δ 7.69 (bs, 1 H), 7.09 (d, *J* = 8.4 Hz, 1 H), 6.85 (d, *J* = 2.1 Hz, 1 H), 6.58 (dd, *J* = 8.4, 2.2 Hz, 1 H), 6.07 (dd, *J* = 1.9, 0.7 Hz, 1 H), 3.46 (bs, 2 H), 2.68 (t, *J* = 7.5 Hz, 2 H), 1.72 (sext, *J* = 7.5 Hz, 2 H), 1.00 (t, *J* = 7.4 Hz, 3 H).



**2-Amino-*N*-(2-propyl-1*H*-indol-5-yl)nicotinamide (15)**. Prepared according to general procedure B from 2-propyl-1*H*-indol-5-amine and 2-aminonicotinic acid. 2-Amino-*N*-(2-propyl-1*H*-indol-5-yl)nicotinamide (**15,** 0.069 g, 80%) was obtained as a beige solid: Mp 176-179 °C; IR (neat) 3312, 1638, 1616, 1578, 1551, 1540, 1444, 1250, 849, 809, 764 cm$^{-1}$; $^1$H NMR (500 MHz, DMSO-*d*$_6$) δ 10.83 (s, 1 H), 9.95 (s, 1 H), 8.10 (dd, *J* = 4.6, 1.2 Hz, 1 H), 8.04 (dd, *J* = 7.5, 0.9 Hz, 1 H), 7.74 (s, 1 H), 7.22 (s, 2 H), 6.96 (bs, 2 H), 6.65 (dd, *J* = 7.6, 4.8 Hz, 1 H), 6.12 (s, 1 H), 2.68 (t, *J* = 7.5 Hz, 2 H), 1.70 (sext, *J* = 7.4 Hz, 2 H), 0.94 (t, *J* = 7.3 Hz, 3 H); $^{13}$C NMR (125 MHz, DMSO-*d*$_6$) δ 166.1, 158.8, 151.1, 140.9, 136.8, 133.2, 130.3, 128.1, 115.3, 112.0, 111.4, 110.7, 110.1, 98.4, 29.8, 22.0, 13.7; HRMS (ESI$^+$) *m/z* calcd for $C_{17}H_{19}N_4O$ $[M+H]^+$ 295.1553, found 295.1552; ELS purity (100%).

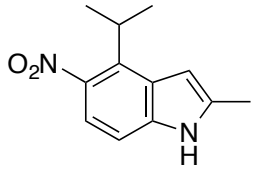


**2-Amino-*N*-(1*H*-indol-5-yl)nicotinamide (16)** Prepared according to general procedure B from 1*H*-indol-5-amine and 2-aminonicotinic acid. 2-Amino-*N*-(1*H*-indol-5-yl)nicotinamide (**16**, 0.162 g, 89%) was obtained as a brown solid: Mp 262-264 °C; IR (neat) 3476, 3346, 3221, 3051, 1633, 1594, 1584, 1571, 1547, 1471, 1446, 1431, 1413, 1340, 1253, 1085, 779, 774, 762, 753, 731, 719 $cm^{-1}$; $^1H$ NMR (400 MHz, DMSO-$d_6$) δ 11.05 (s, 1 H), 10.03 (s, 1 H), 8.12 (d, $J$ = 2.4 Hz, 1 H), 8.07 (d, $J$ = 7.3 Hz, 1 H), 7.91 (s, 1 H), 7.38-7.34 (m, 3 H), 6.99 (bs, 2 H), 6.66 (dd, $J$ = 6.7, 5.0 Hz, 1 H), 6.42 (s, 1 H); $^{13}C$ NMR (100 MHz, DMSO-$d_6$) δ 166.2, 158.8, 151.1, 136.9, 133.1, 130.6, 127.4, 125.9, 116.4, 112.7, 111.4, 111.0, 110.7, 101.2; HRMS ($ESI^+$) *m/z* calcd for $C_{14}H_{13}N_4O$ $[M+H]^+$ 253.1084, found 253.1084; ELS purity (100%).

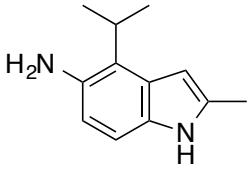


**2-Amino-*N*-(2,4-dimethyl-1*H*-indol-5-yl)nicotinamide (17)** Prepared according to general procedure C from 2,4-dimethyl-1*H*-indol-5-amine[4] and 2-aminonicotinic acid. 2-Amino-*N*-(2,4-dimethyl-1*H*-indol-5-yl)nicotinamide (**17**, 0.041 g, 31%) was obtained as an off-white solid: Mp 188-190 °C; IR (neat) 3422, 3303, 3058, 2975, 1675, 1629, 1575, 1474, 1450, 1232, 839, 768, 675 $cm^{-1}$; $^1H$ NMR (400 MHz, DMSO-$d_6$) δ 10.88 (s, 1 H), 9.76 (s, 1 H), 8.14-8.10 (m, 2 H), 7.09 (d, $J$ = 8.4 Hz, 1 H), 7.03 (bs, 2 H), 6.85 (d, $J$ = 8.4 Hz, 1 H), 6.64 (dd, $J$ = 7.6, 4.9 Hz, 1 H), 6.17 (d, $J$ = 0.9 Hz, 1 H), 2.39 (s, 3 H), 2.27 (s, 3 H); $^{13}C$ NMR (100 MHz, DMSO-$d_6$) δ 166.8, 159.0, 151.2, 136.8, 135.4, 134.1, 129.0, 126.5, 124.2, 120.1, 111.4, 109.9, 107.8, 98.3, 14.3, 13.5; HRMS ($ESI^+$) *m/z* calcd for $C_{16}H_{17}N_4O$ $[M+H]^+$ 281.1397, found 281.1395; ELS purity (100%).

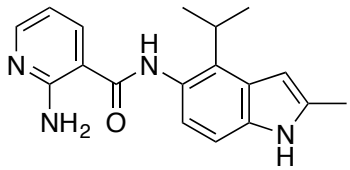
[4] Synthesis of **2,4-dimethyl-1*H*-indol-5-amine** according to Niu, C.; Boschelli, D.H.; Tumey, L.N.; Bhagirath, N.; Subrath, J.; Shim, J.; Wang. Y.; Wu, b.; Eid, C.; Lee, J.; Yang, X.; Brennan, A.; Chaudhary, D. *Bioorg. Med. Chem. Lett.* **2009**, *19*, 5829-5832.



**4-Isopropyl-2-methyl-5-nitro-1*H*-indole.** Synthesized according to literature precedence.[4] 4-Isopropyl-2-methyl-5-nitro-1*H*-indole (0.157 g, 48%) was obtained as a dark amber oil: IR (neat) 3380, 2974, 2950, 1610, 1550, 1505, 1485, 1325, 1160, 1110, 805, 770, 720 $cm^{-1}$; $^1H$ NMR (500 MHz, $CDCl_3$) δ 8.45 (bs, 1 H), 7.45 (d, *J* = 8.7 Hz, 1 H), 7.12 (dd, *J* = 8.7, 0.7 Hz, 1 H), 6.54 (app t, *J* = 0.9 Hz, 1 H), 3.66 (sept, *J* = 7.1 Hz, 1 H), 2.47 (s, 3 H), 1.50 (d, *J* = 7.1 Hz, 6 H); $^{13}C$ NMR (125 MHz, $CDCl_3$) δ 143.5, 137.9, 137.1, 134.9, 126.7, 117.5, 108.6, 102.6, 29.5, 22.0, 13.8; HRMS ($ESI^+$) *m/z* calcd for $C_{12}H_{15}N_2O_2$ $[M+H]^+$ 219.1128, found 219.1126.

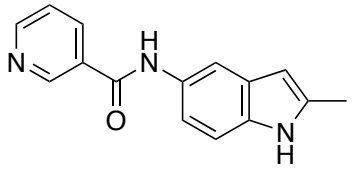


**4-Isopropyl-2-methyl-1*H*-indol-5-amine.** Synthesized according to literature precedence.[4] 4-Isopropyl-2-methyl-1*H*-indol-5-amine (0.122 g, 90%, includes ca. 20% of solvent impurities) was obtained as a red-brown solid: IR (neat) 3295, 2974, 2868, 1593, 1450, 1420, 1410, 1358, 1340, 1232, 1163,839, 798, 768 $cm^{-1}$; $^1H$ NMR (500 MHz, $CDCl_3$) δ 7.69 (bs, 1 H), 6.95 (d, *J* = 8.3 Hz, 1 H), 6.57 (d, *J* = 8.3 Hz, 1 H), 6.27 (app s, 1 H), 3.84 (bs, 2 H), 3.35 (sept, *J* = 8.0 Hz, 1 H), 2.40 (s, 3 H), 1.45 (d, *J* = 7.1 Hz, 6 H); $^{13}C$ NMR (125 MHz, $CDCl_3$) δ 135.2, 134.4, 131.9, 127.7, 123.2, 113.4, 108.7, 100.0, 28.3, 21.6, 13.9; HRMS ($ESI^+$) *m/z* calcd for $C_{12}H_{17}N_2$ $[M+H]^+$ 189.1386, found 189.1385.

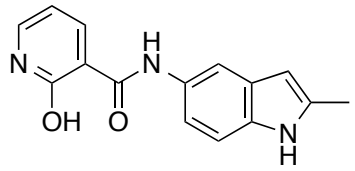


**2-Amino-*N*-(4-isopropyl-2-methyl-1*H*-indol-5-yl)nicotinamide (18).** Prepared according to general procedure C from 4-isopropyl-2-methyl-1*H*-indol-5-amine and 2-

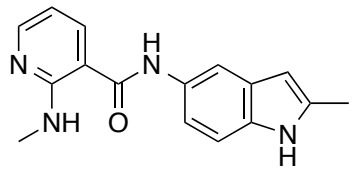
aminonicotinic acid. 2-Amino-*N*-(4-isopropyl-2-methyl-1*H*-indol-5-yl)nicotinamide (**18**, 0.047 g, 46%, includes ca. 20% of solvent impurities) was obtained as an off-white solid: Mp 193-196 °C; IR (neat) 3435, 3403, 3303, 2934, 2975, 2934, 1630, 1554, 1450, 1192, 1163, 1094, 839, 768 $cm^{-1}$; $^{1}H$ NMR (500 MHz, DMSO-$d_6$) δ 10.90 (s, 1 H), 9.74 (s, 1 H), 8.14-8.11 (m, 2 H), 7.09 (d, $J$ = 8.3 Hz, 1 H), 7.04 (bs, 2 H), 6.75 (d, $J$ = 8.1 Hz, 1 H), 6.64 (dd, $J$ = 5.5, 5.5 Hz, 1 H), 6.32 (s, 1 H), 3.36-3.29 (m, 1 H, partial overlap with water peak), 2.39 (s, 3 H), 1.33 (d, $J$ = 6.9 Hz, 6 H); $^{13}C$ NMR (125 MHz, DMSO-$d_6$) δ 167.5, 159.0, 151.2, 136.7, 135.6, 135.2, 134.9, 126.0, 125.0, 121.2, 111.4, 109.8, 108.4, 99.5, 28.4, 21.7, 13.4; HRMS ($ESI^+$) *m/z* calcd for $C_{18}H_{21}N_4O$ $[M+H]^+$ 309.1710, found 309.1709; ELS purity (99.7%).




***N*-(2-Methyl-1*H*-indol-5-yl)nicotinamide (19).** Prepared according to general procedure A from 2-methyl-1*H*-indol-5-amine and nicotinic acid. *N*-(2-Methyl-1*H*-indol-5-yl)nicotinamide (**19**, 0.13 g, 52%, includes ca. 15% aliphatic impurities) was obtained as a pale blue solid: Mp 203-204 °C; IR (neat) 3301, 3133, 1636, 1536, 1325, 1279, 783 $cm^{-1}$; $^{1}H$ NMR (500 MHz, DMSO-$d_6$) δ 10.87 (bs, 1 H), 10.21 (s, 1 H), 9.11 (d, $J$ = 1.7 Hz, 1 H), 8.74 (dd, $J$ = 4.8, 1.6 Hz, 1 H), 8.29 (dt, $J$ = 8.0, 2.0 Hz, 1 H), 7.84 (d, $J$ = 1.6 Hz, 1 H), 7.55 (ddd, $J$ = 7.9, 4.8, 0.6 Hz, 1 H), 7.29 (dd, $J$ = 8.6, 1.9 Hz, 1 H), 7.23 (d, $J$ = 8.6 Hz, 1 H), 6.11 (app t, $J$ = 0.9 Hz, 1 H), 2.37 (s, 3 H); $^{13}C$ NMR (125 MHz, DMSO-$d_6$) δ 163.4, 151.7, 148.5, 136.3, 135.2, 133.3, 131.0, 130.4, 128.4, 123.4, 114.7, 111.3, 110.1, 99.3, 13.4; HRMS ($ESI^+$) *m/z* calcd for $C_{15}H_{14}N_3O$ $[M+H]^+$ 252.1131, found 252.1128; ELS purity (100%).



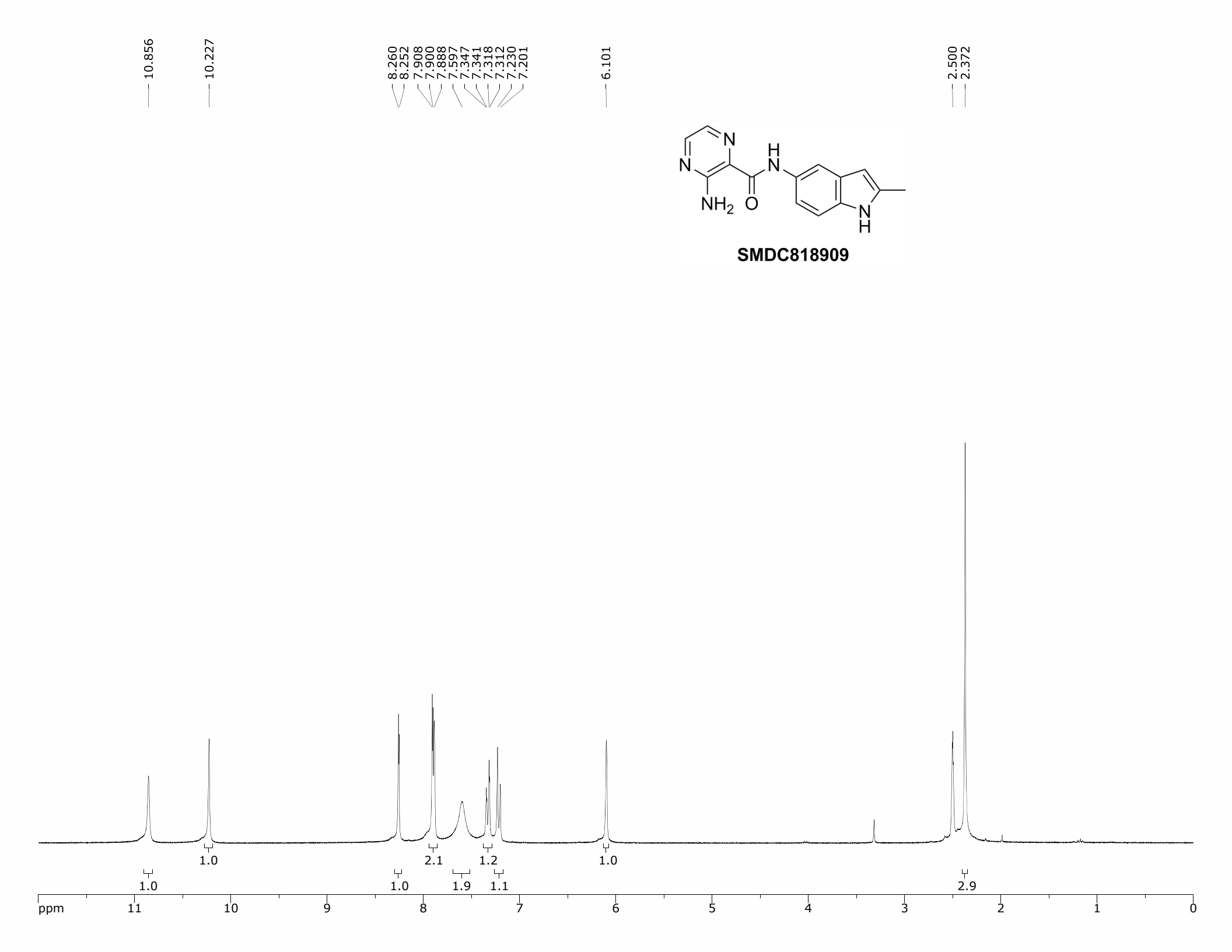
**2-Hydroxy-*N*-(2-methyl-1*H*-indol-5-yl)nicotinamide (20).** To a solution of 2-hydroxynicotinic acid (0.100 g, 0.719 mmol) in DMF (2.2 mL), TEA (0.29 g, 2.9 mmol, 0.40 mL) and HATU (0.328 g, 0.863 mmol) were added. The mixture was stirred at room temperature for 5 min prior to the addition of 2-methyl-1*H*-indol-5-amine (0.105 mg, 0.719 mmol). The reaction mixture was stirred for 12 h at room temperature under inert atmosphere. The resulting mixture was diluted with EtOAc, and the organic phase was washed with $H_2O$ and brine, dried ($Na_2SO_4$), and concentrated. The residue was adsorbed onto $SiO_2$ and purified by chromatography on $SiO_2$ (EtOAc/hexanes, 80-100%) to give 2-hydroxy-*N*-(2-methyl-1*H*-indol-5-yl)nicotinamide (**20**, 0.101 g, 52%) as a tan solid: Mp 280-282 °C (dec); IR (neat) 3286, 2837, 1659, 1592, 1575, 1545, 1476, 1245, 761, 662 $cm^{-1}$; $^1H$ NMR (400 MHz, DMSO-$d_6$) δ 12.66 (bs, 1 H), 12.01 (s, 1 H), 10.87 (s, 1 H), 8.46 (dd, $J$ = 6.9, 1.3 Hz, 1 H), 7.87 (s, 1 H), 7.78-7.77 (m, 1 H), 7.22 (d, $J$ = 8.7 Hz, 1 H), 7.15 (d, $J$ = 8.2 Hz, 1 H), 6.56 (t, $J$ = 6.6 Hz, 1 H), 6.09 (s, 1 H), 2.36 (s, 3 H); $^{13}C$ NMR (100 MHz, DMSO-$d_6$) δ 162.6, 160.8, 144.0, 139.5, 136.5, 133.1, 130.4, 128.7, 120.7, 113.7, 110.6, 110.1, 106.8, 99.3, 13.4; HRMS ($ESI^+$) *m/z* calcd for $C_{15}H_{14}N_3O_2$ $[M+H]^+$ 268.1081, found 268.1074; ELS purity (100%).



***N*-(2-Methyl-1*H*-indol-5-yl)-2-(methylamino)nicotinamide (21)** Prepared according to general procedure B from 2-methyl-1*H*-indol-5-amine and 2-(methylamino)nicotinic acid.[5] *N*-(2-Methyl-1*H*-indol-5-yl)-2-(methylamino)nicotinamide (**21**, 0.074 g, 62%) was obtained as a brown solid: Mp 216-220 °C; IR (neat) 3271, 1630, 1523, 1261, 781, 769 $cm^{-1}$; $^1H$ NMR (400 MHz, DMSO-$d_6$) δ 10.86 (bs, 1 H), 9.99 (s, 1 H), 8.20 (d, $J$ = 4.3 Hz, 1 H), 8.04 (d, $J$ = 7.4 Hz, 1 H), 7.95 (app d, $J$ = 4.2 Hz, 1 H), 7.75 (s, 1 H), 7.22 (bs, 2 H),

[5] Synthesis of **2-(methylamino)nicotinic acid** according to Quevedo, C.E.; Bavetsias, V.; McDonald, E. *Tetrahedron Lett*. **2009**, *50*, 2481-2483.

6.62 (dd, $J$ = 7.1, 5.1 Hz, 1 H), 6.10 (s, 1 H), 2.91 (d, $J$ = 4.6 Hz, 3 H), 2.37 (s, 3 H); $^{13}$C NMR (100 MHz, DMSO-$d_6$) δ 166.3, 157.9, 150.9, 136.5, 136.3, 133.3, 130.3, 128.4, 115.3, 111.9, 111.3, 110.2, 110.0, 99.2, 27.7, 13.5; HRMS ($ESI^+$) *m/z* calcd for $C_{16}H_{17}N_4O$ $[M+H]^+$ 281.1397, found 281.1394; ELS purity (99.6%).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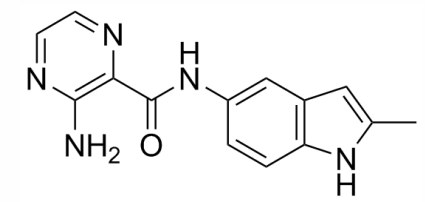


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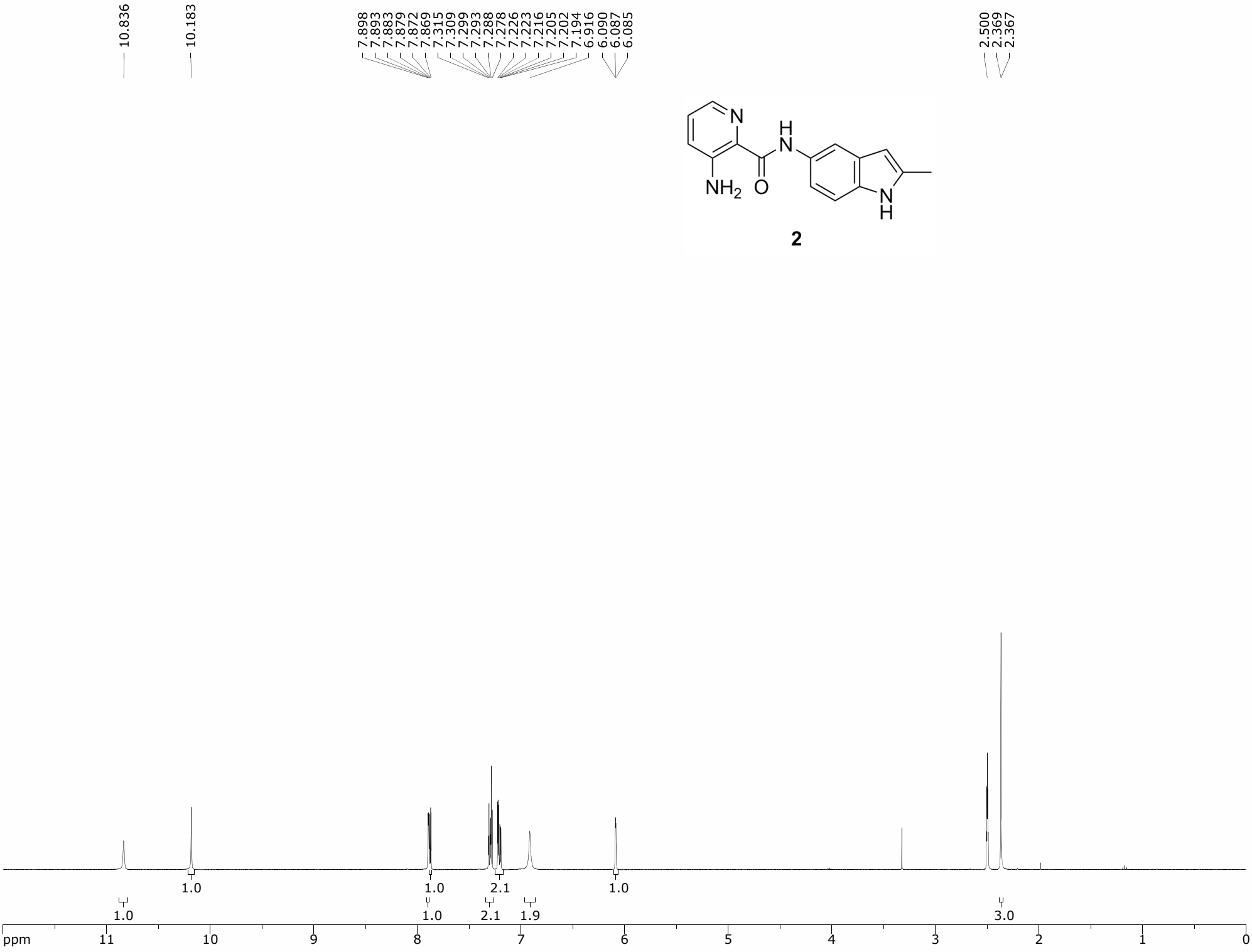
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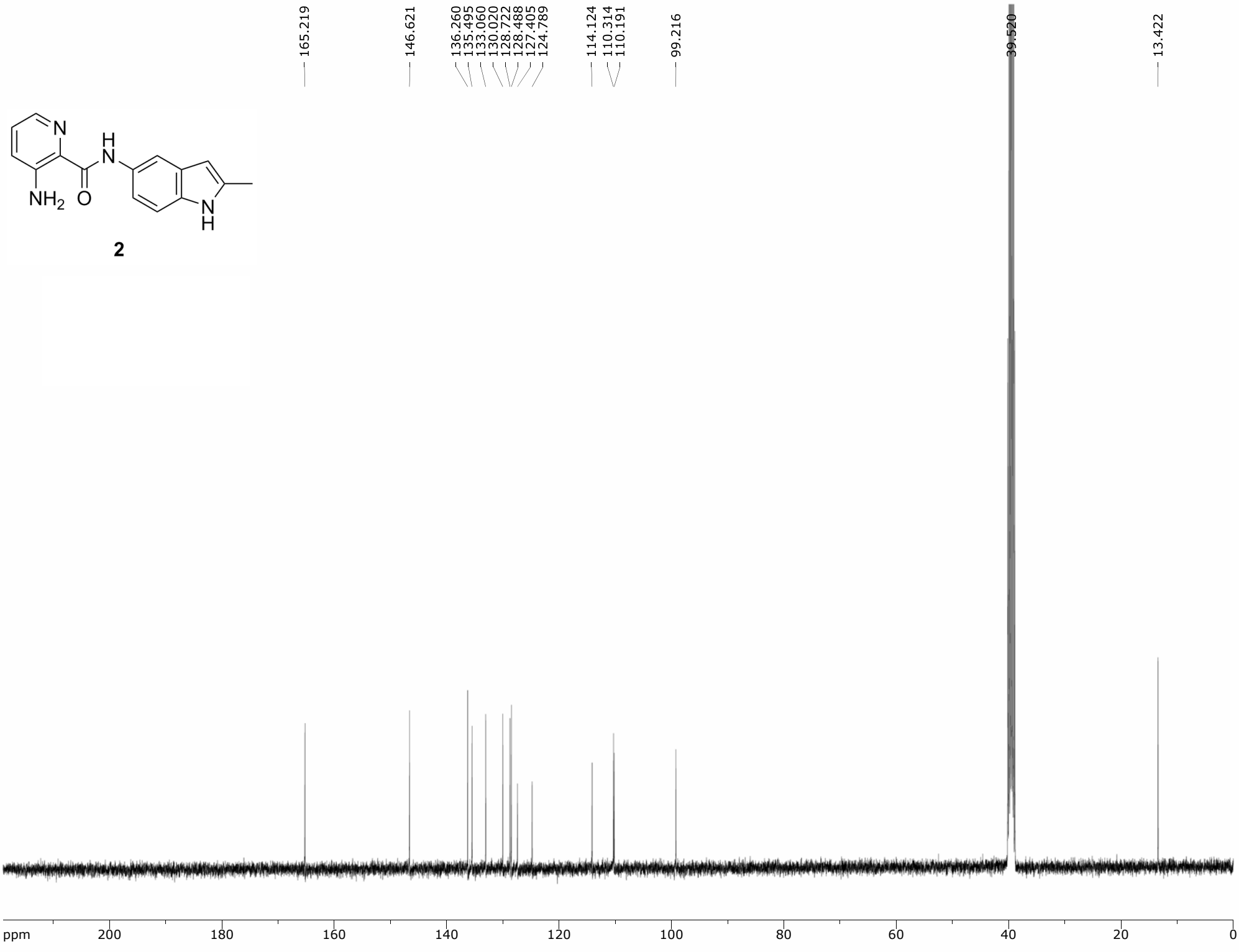
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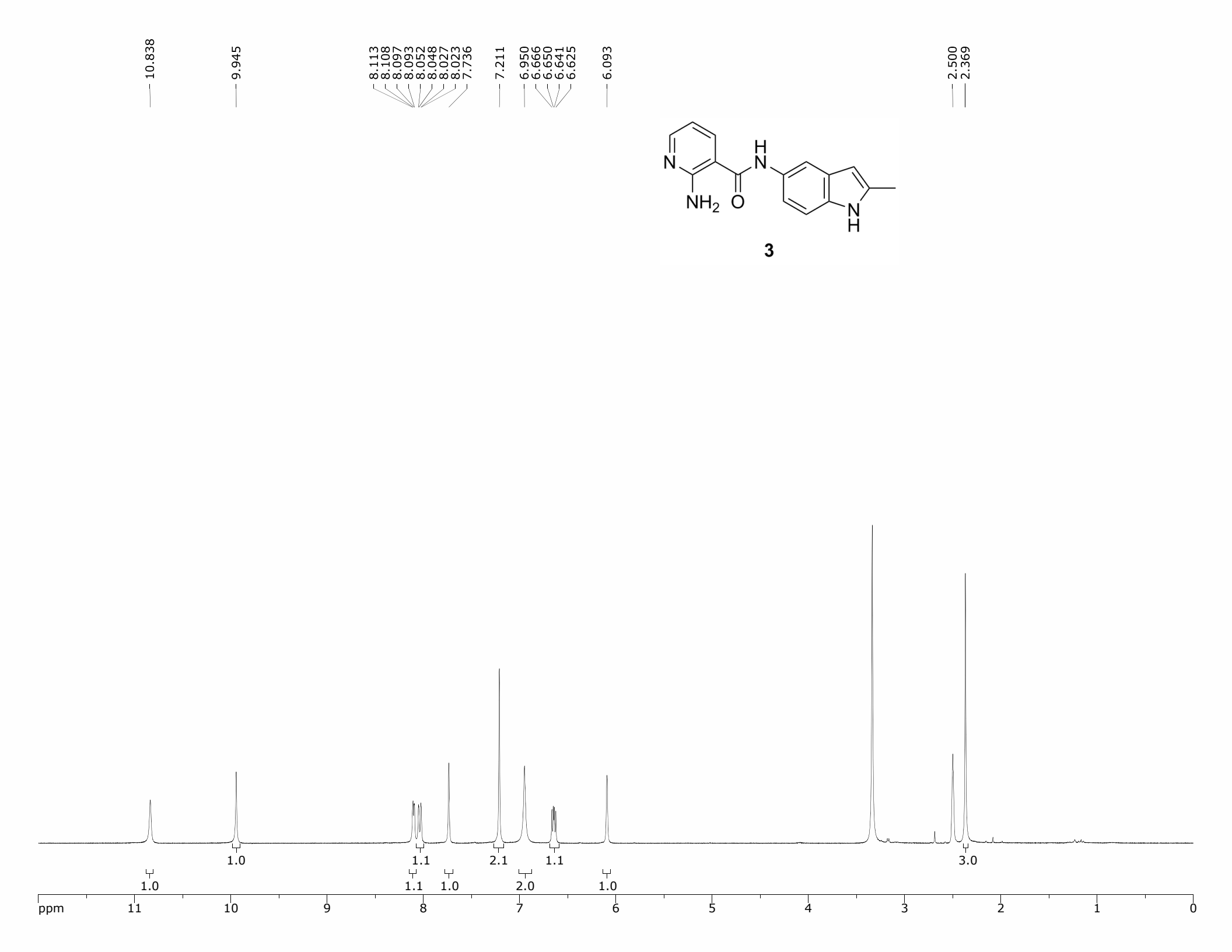
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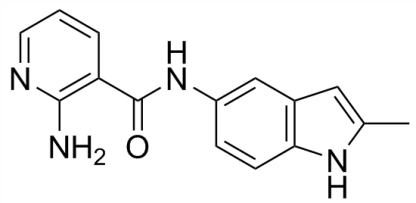

**3**

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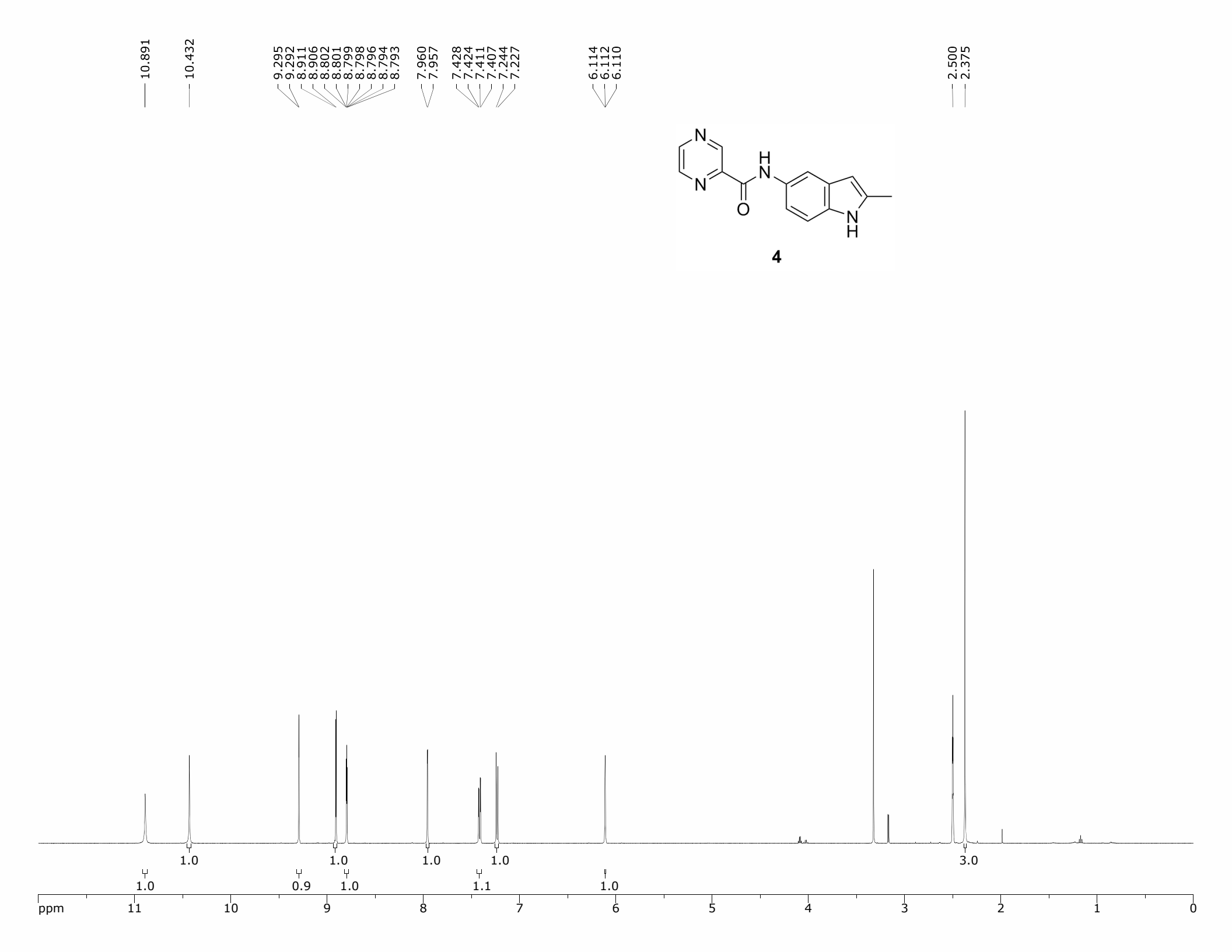
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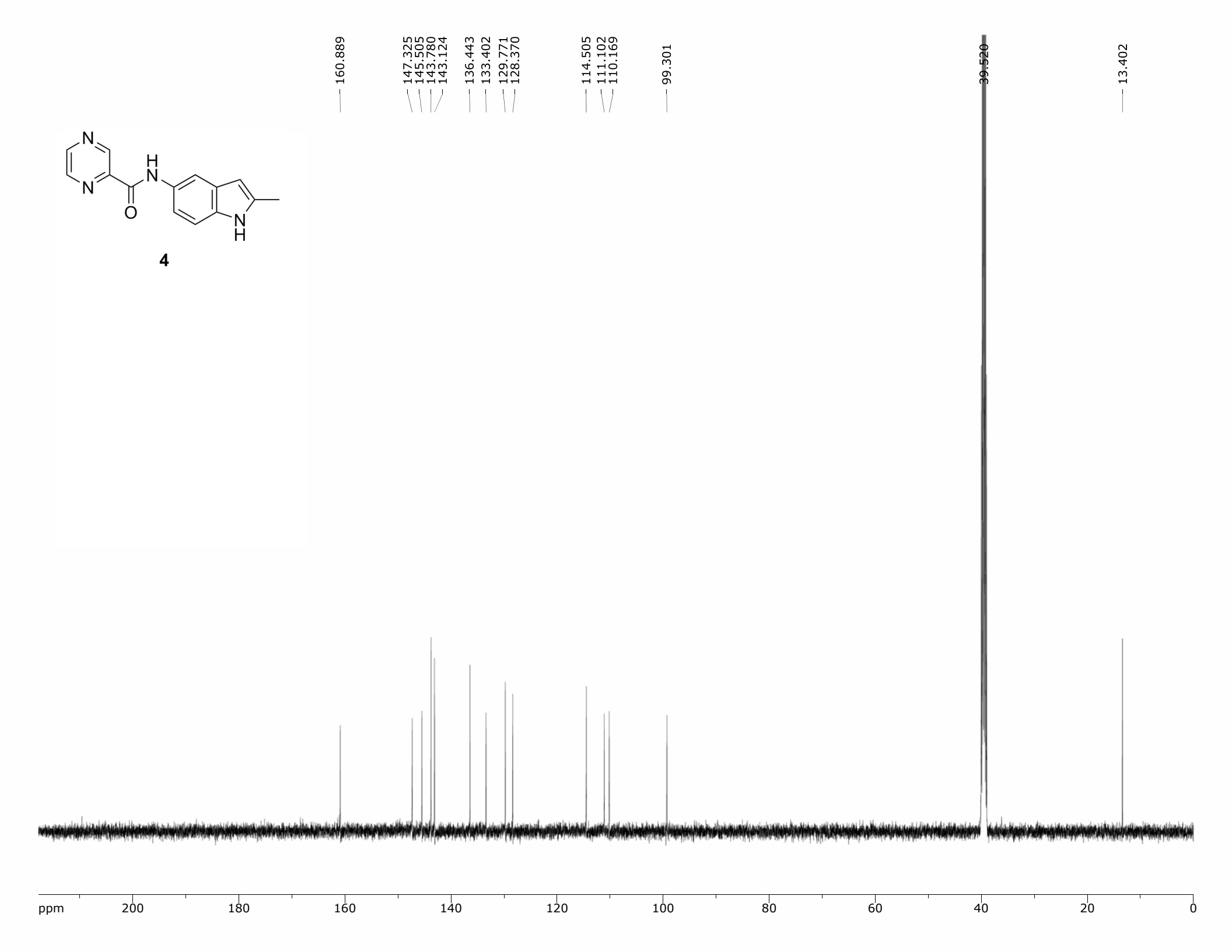
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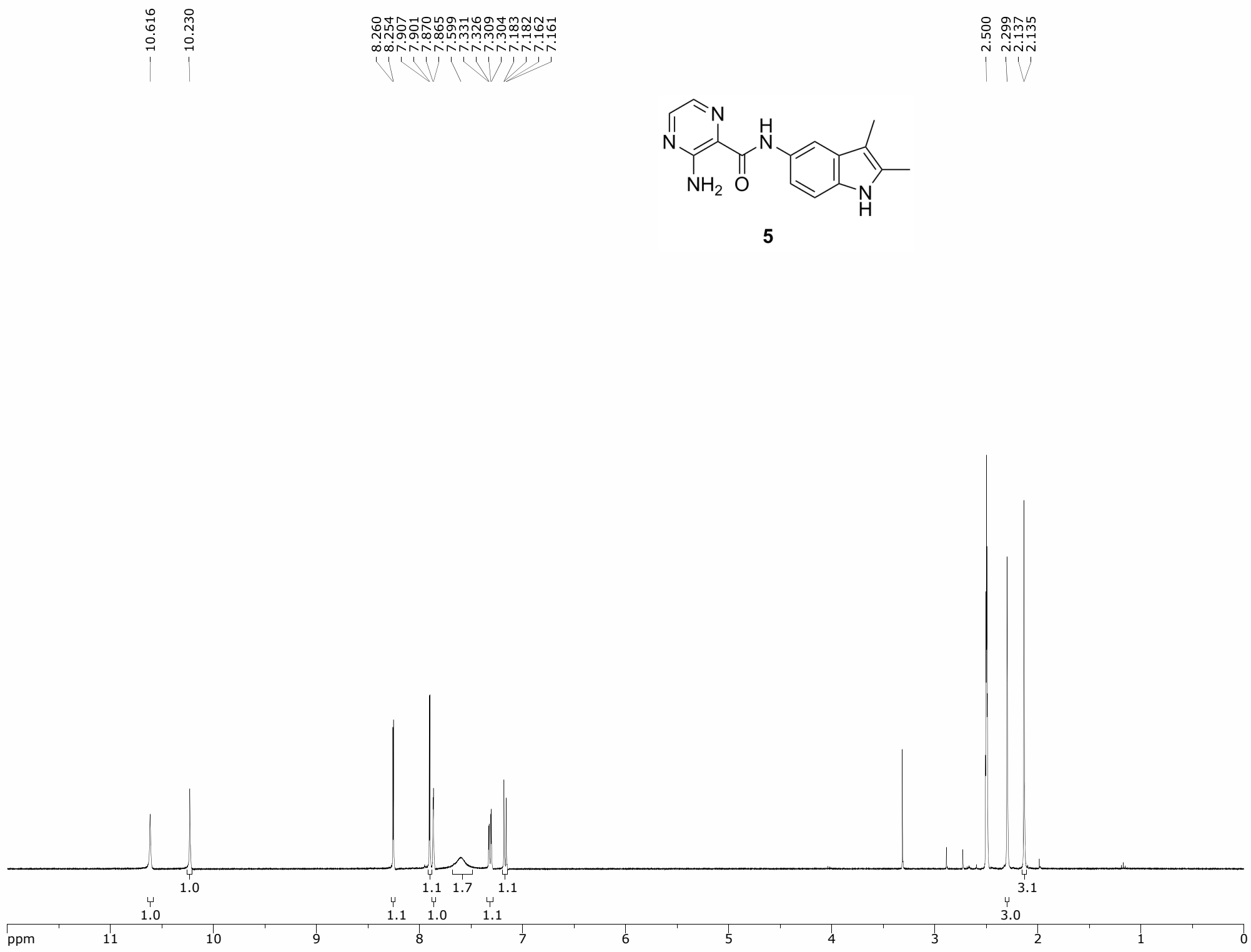
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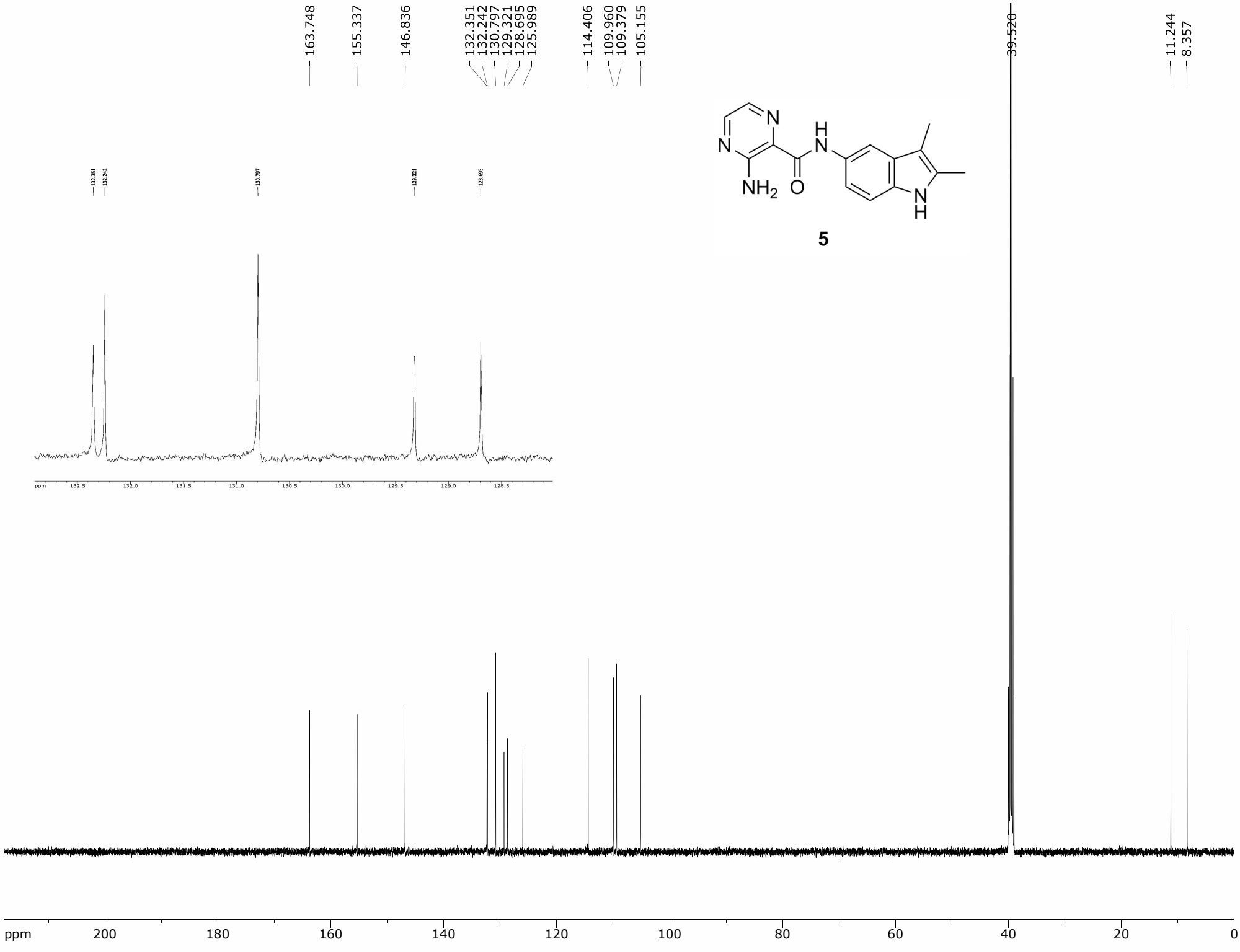
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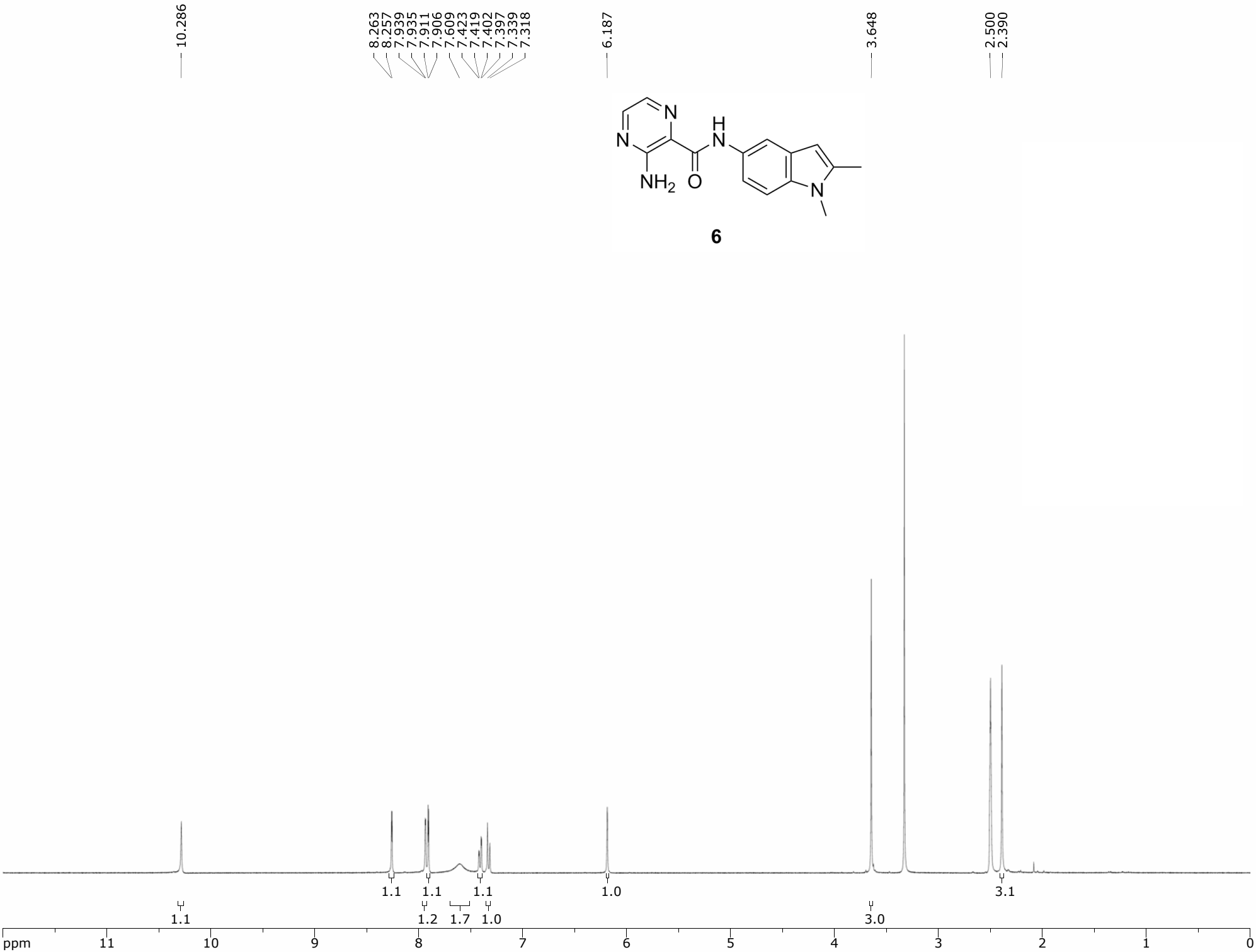
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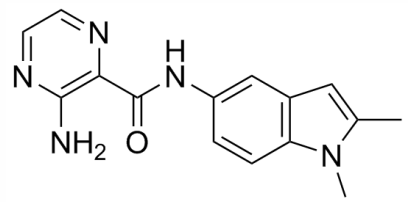
**6**



**6**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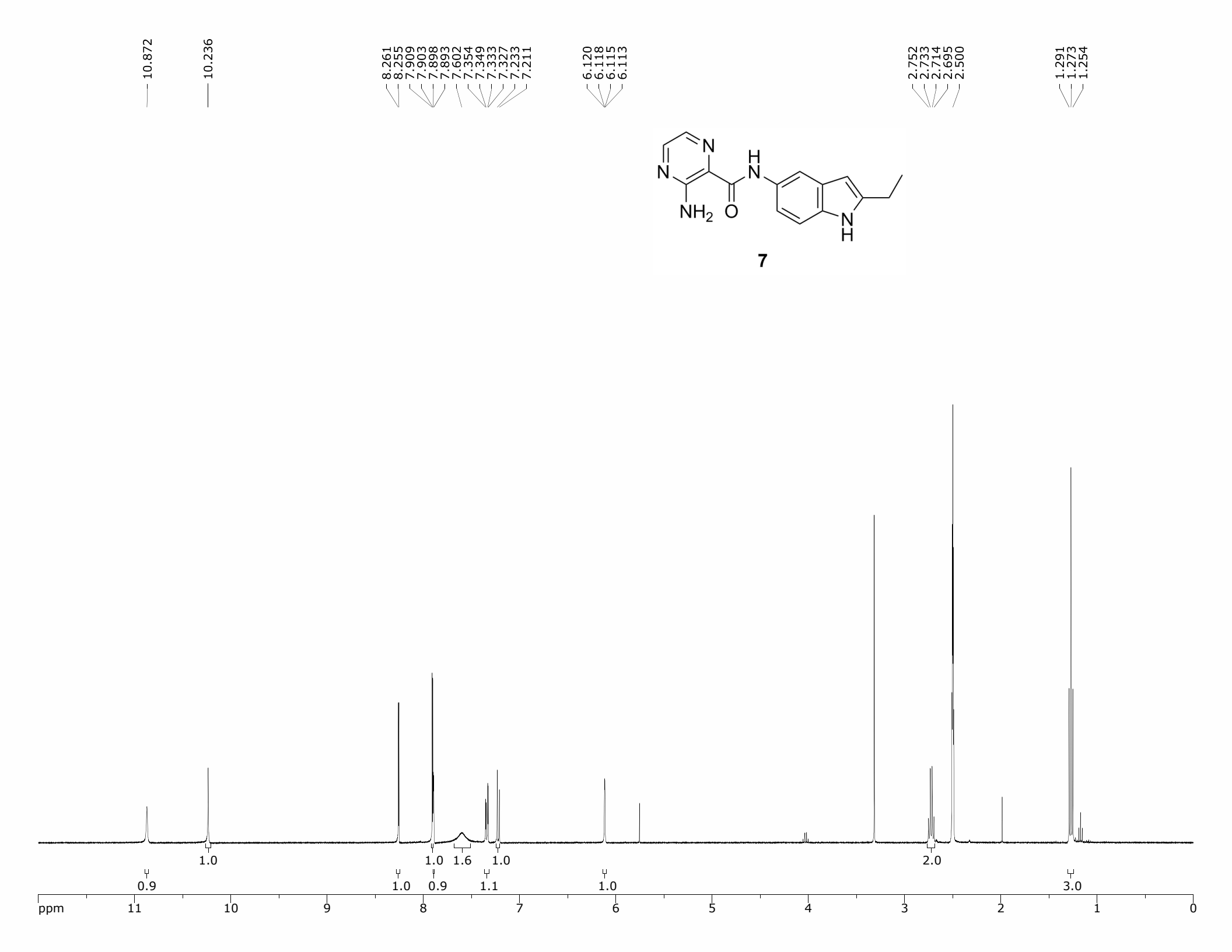
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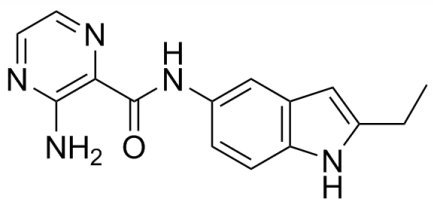

**7**


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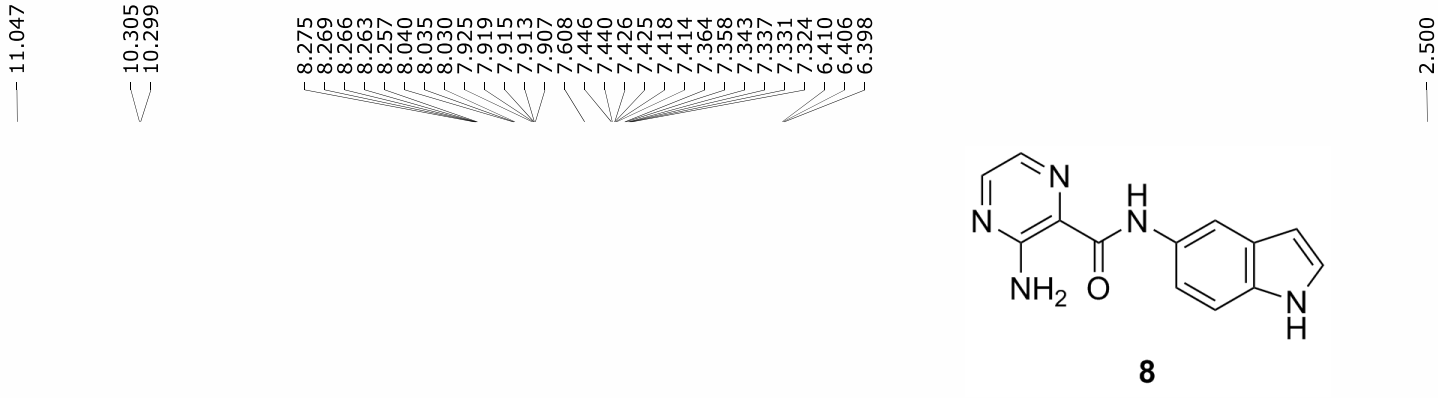
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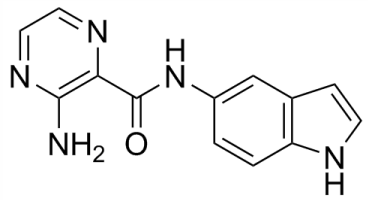

**8**


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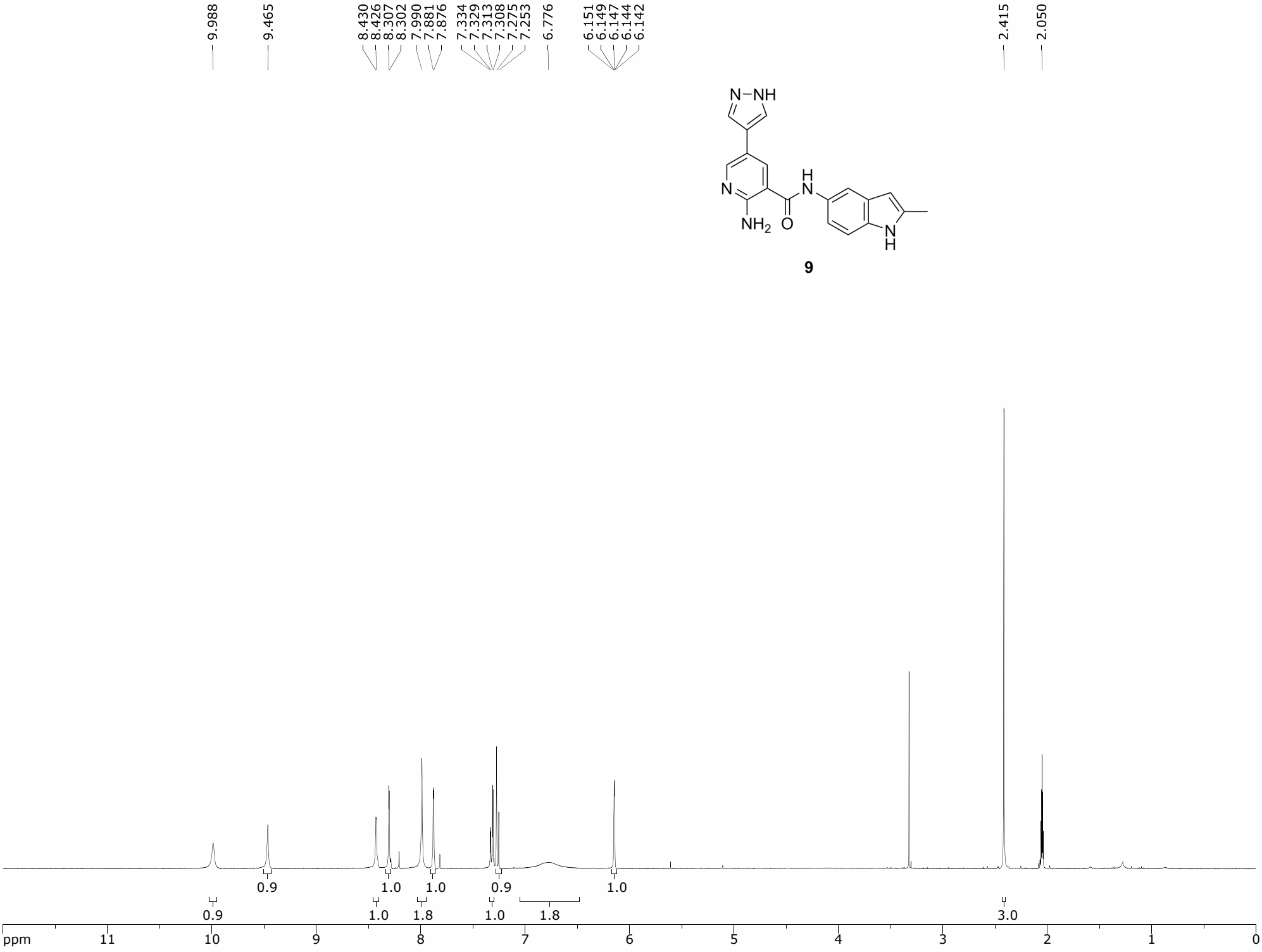
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7.308
7.275
7.253
6.776
6.151
6.149
6.147
6.144
6.142
2.415
2.050
N–NH
N
H
N
NH2
O
N
H
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1.0
0.9
1.0
0.9
1.0
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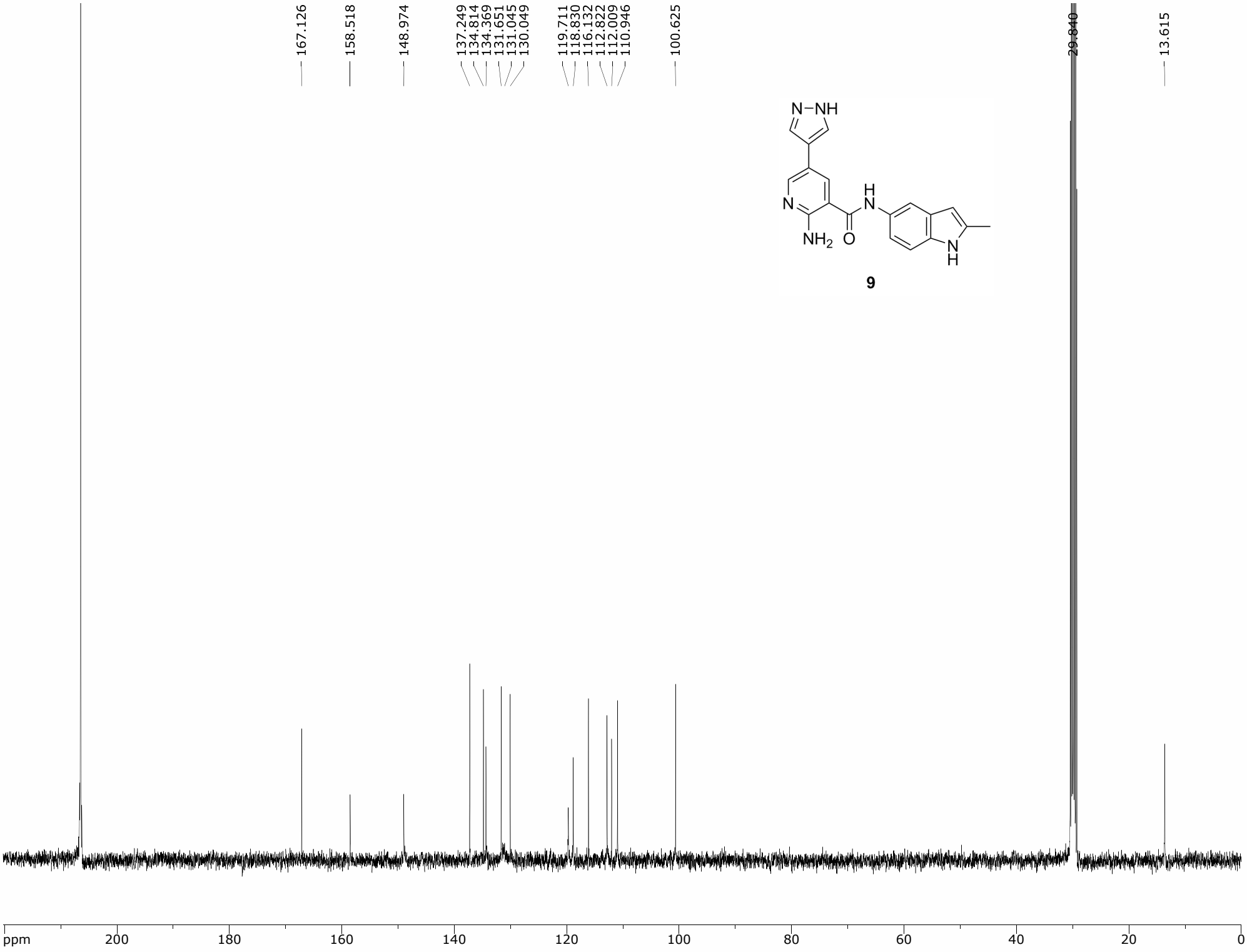
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137.249
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131.045
130.049
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116.132
112.822
112.009
110.946
100.625
29.840
13.615
N–NH
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H
N
NH2
O
N
H
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ppm
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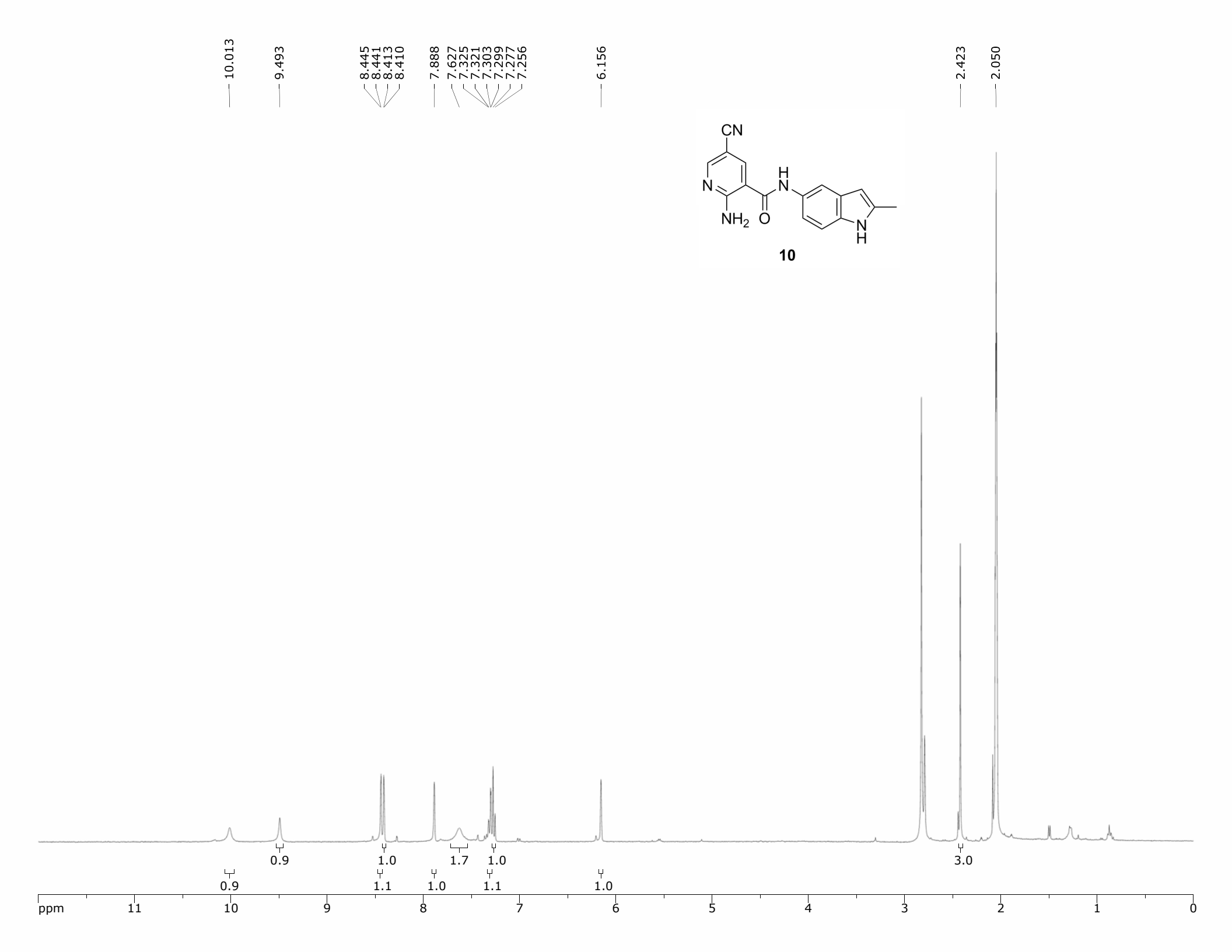
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8.410
7.888
7.627
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7.321
7.303
7.299
7.277
7.256
6.156
2.423
2.050
CN
N
H
N
NH2
O
N
H
10
0.9
1.0
1.7
1.0
3.0
0.9
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1.0
1.1
1.0
ppm
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10
9
8
7
6
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4
3
2
1
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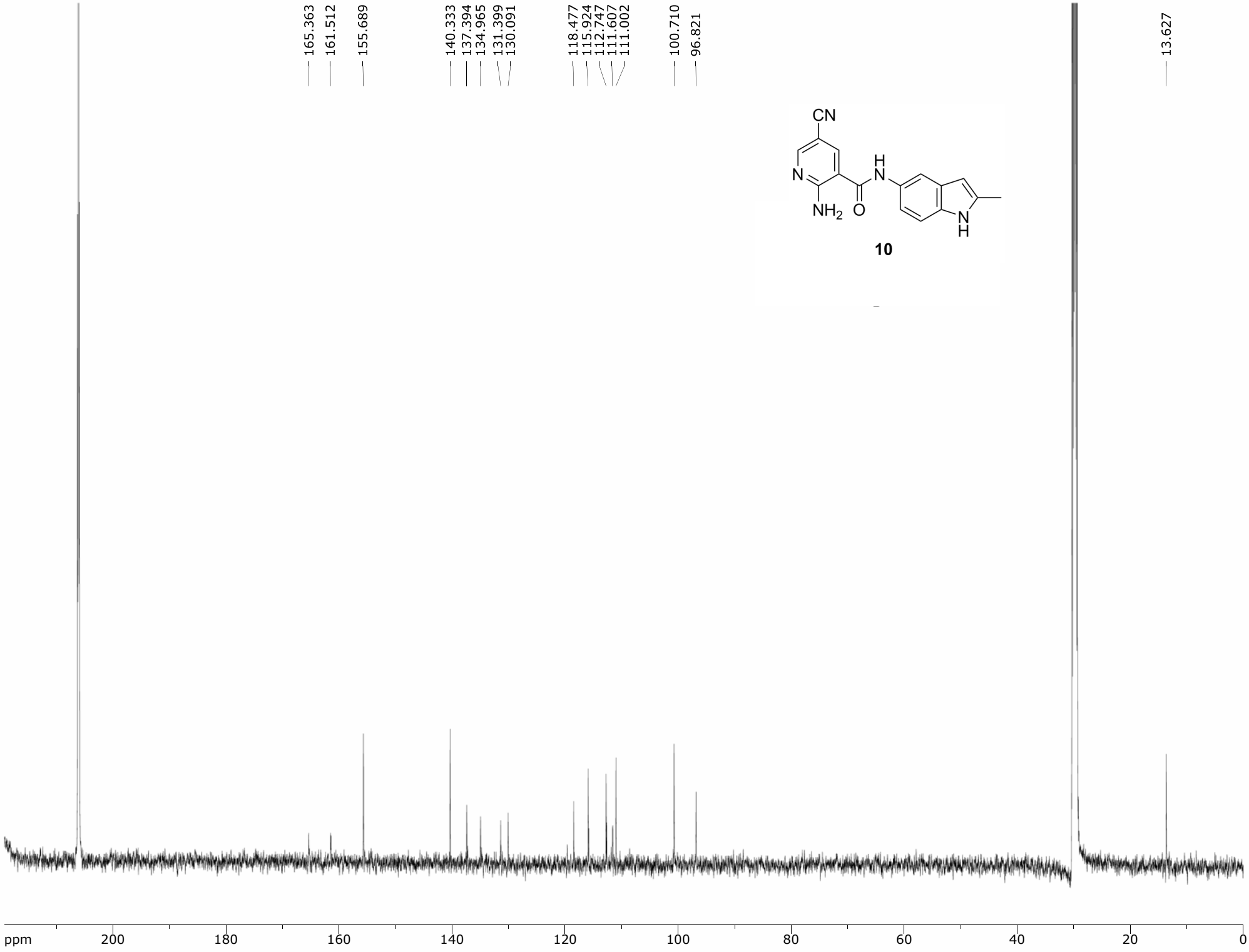
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155.689
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130.091
118.477
115.924
112.747
111.607
111.002
100.710
96.821
13.627
CN
N
H
N
NH2
O
N
H
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ppm
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140
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100
80
60
40
20
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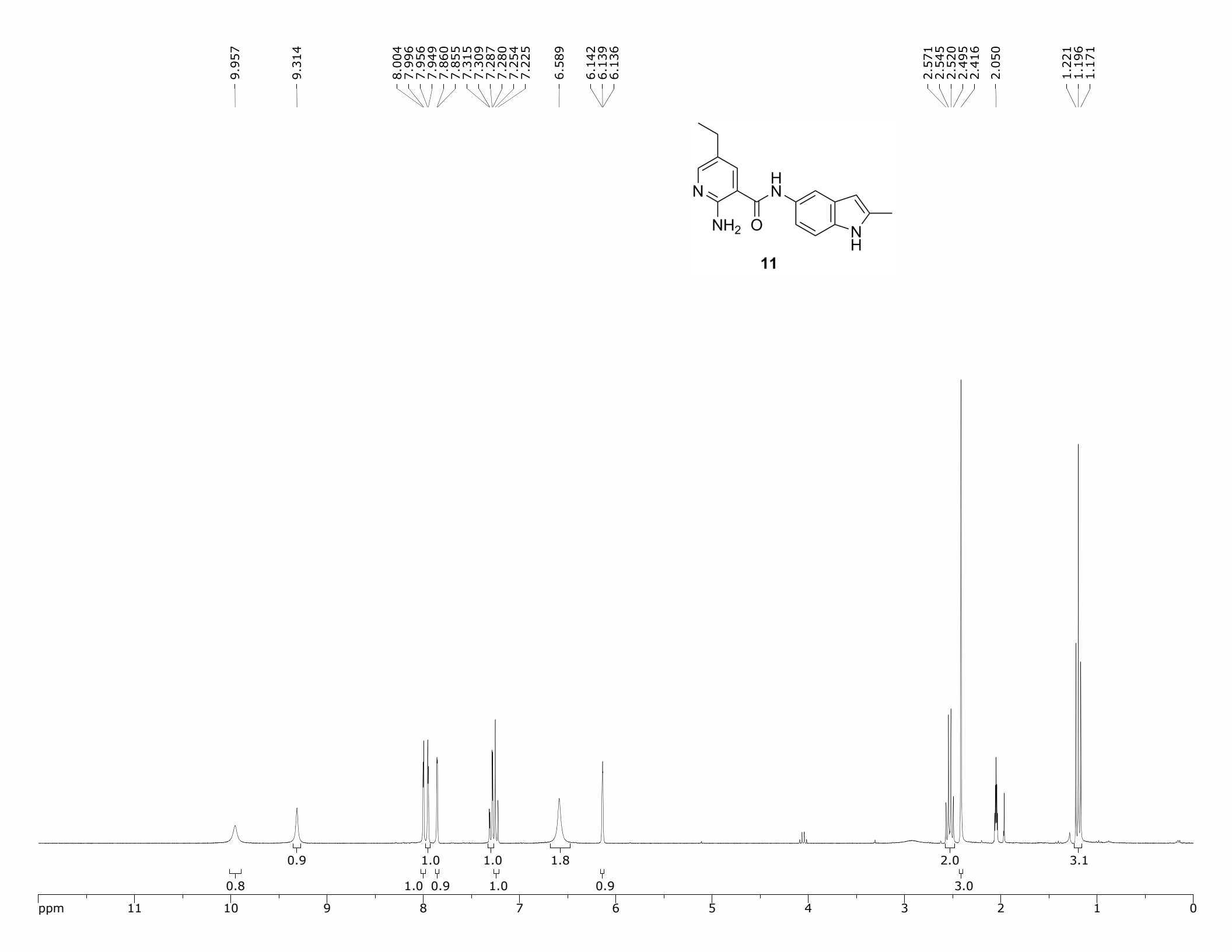
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9.314
8.004
7.996
7.956
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7.855
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7.309
7.287
7.280
7.254
7.225
6.589
6.142
6.139
6.136
2.571
2.545
2.520
2.495
2.416
2.050
1.221
1.196
1.171
N
H
N
NH2
O
N
H
11
0.9
1.0
1.0
1.8
2.0
3.1
0.8
1.0
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1.0
0.9
3.0
ppm
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9
8
7
6
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4
3
2
1
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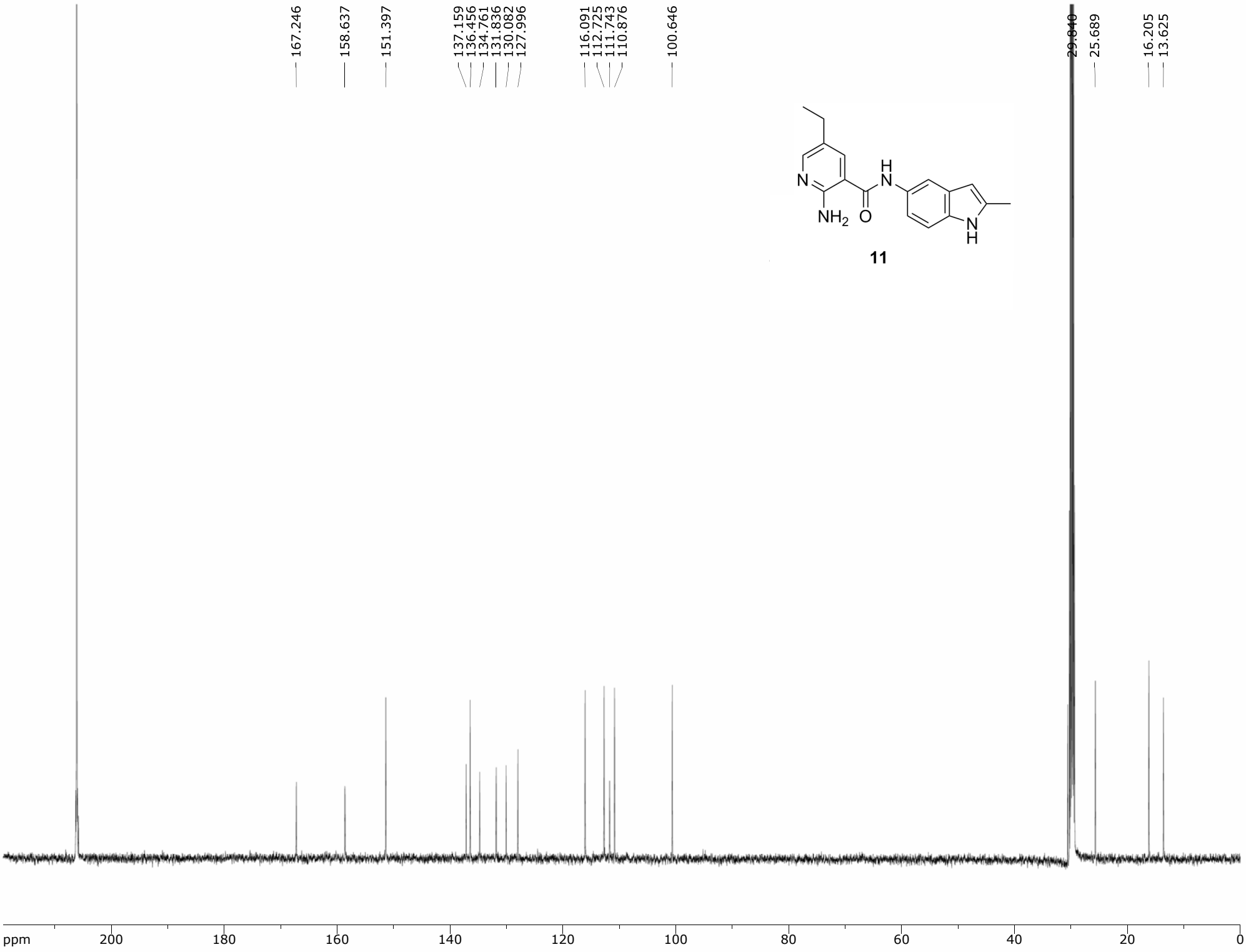
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136.456
134.761
131.836
130.082
127.996
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111.743
110.876
100.646
29.840
25.689
16.205
13.625
N
H
N
NH2
O
N
H
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ppm
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140
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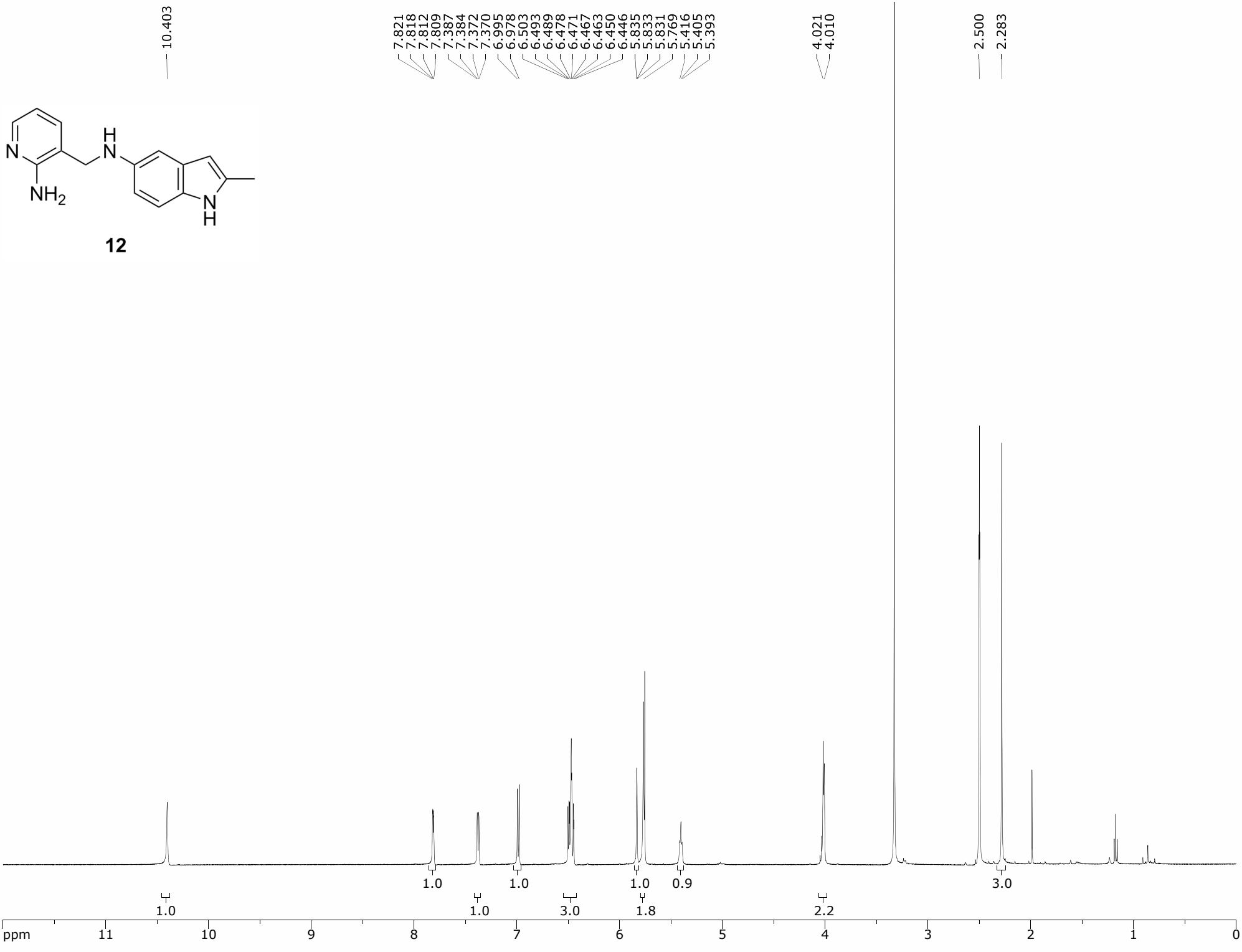
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7.372
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6.978
6.503
6.493
6.489
6.478
6.471
6.467
6.463
6.450
6.446
5.835
5.833
5.831
5.769
5.416
5.405
5.393
4.021
4.010
2.500
2.283
N
H
N
NH2
N
H
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1.0
1.0
0.9
3.0
1.0
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1.8
2.2
ppm
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9
8
7
6
5
4
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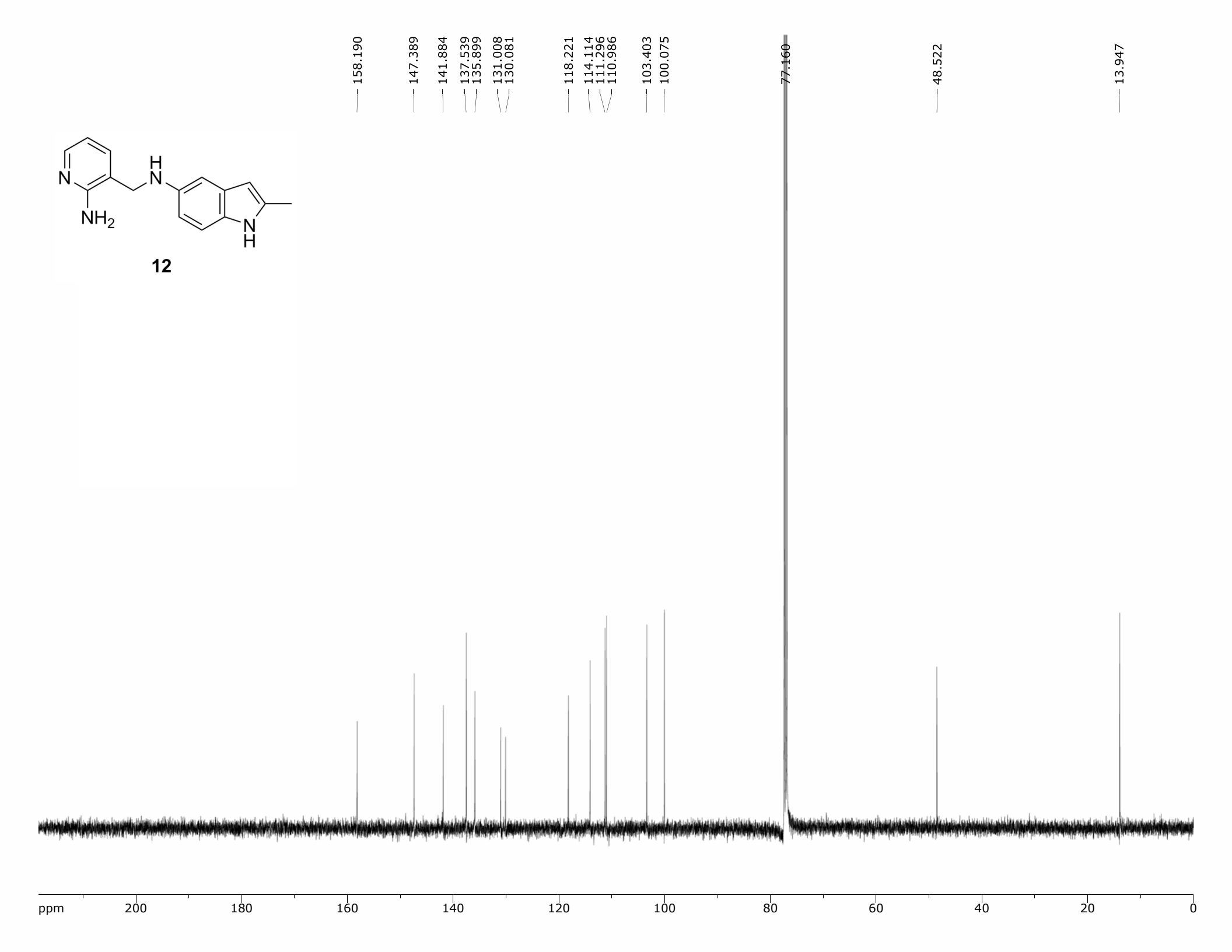
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141.884
137.539
135.899
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130.081
118.221
114.114
111.296
110.986
103.403
100.075
77.160
48.522
13.947
N
H
N
NH2
N
H
12
ppm
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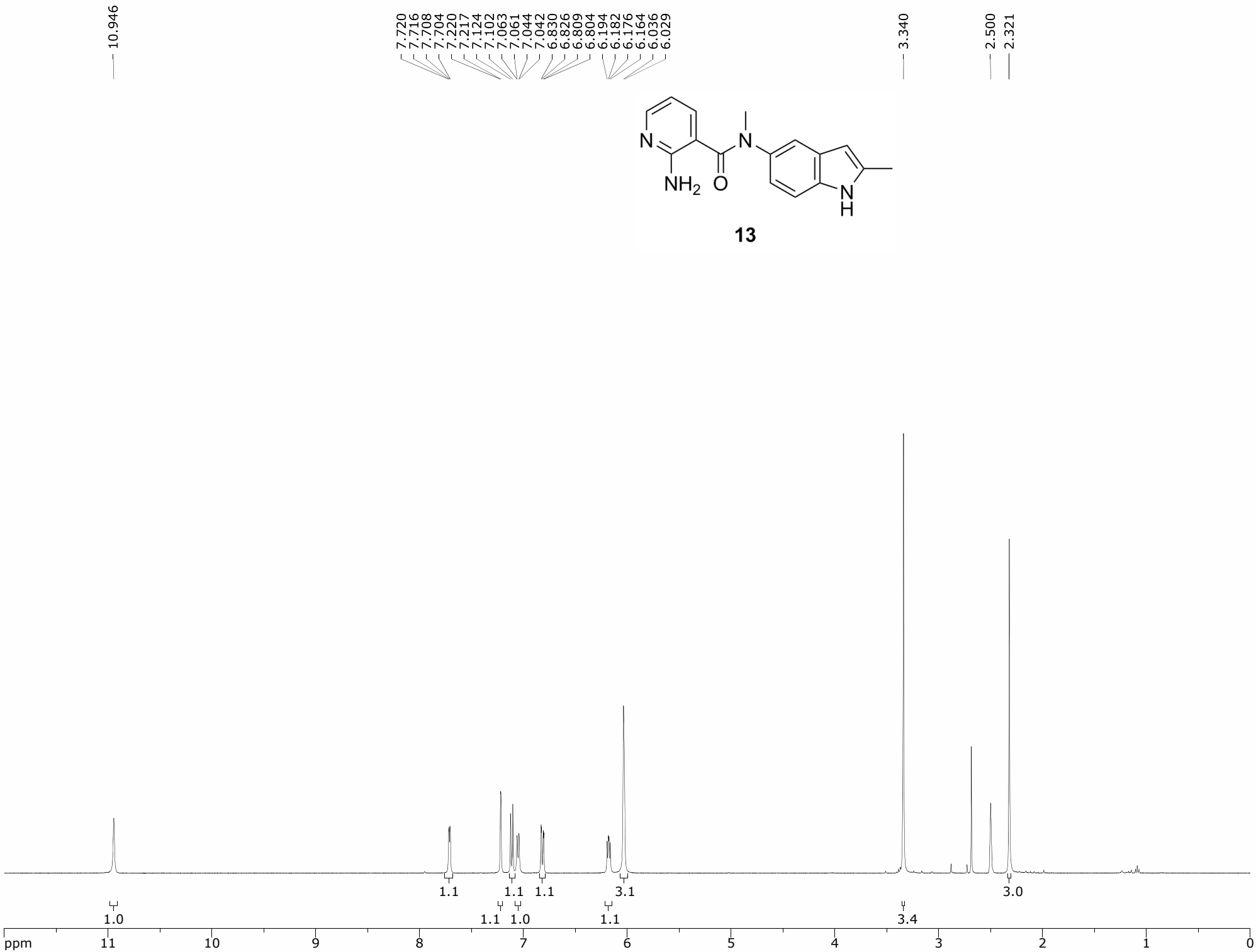
140
120
100
80
60
40
20
0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7.704
7.220
7.217
7.124
7.102
7.063
7.061
7.044
7.042
6.830
6.826
6.809
6.804
6.194
6.182
6.176
6.164
6.036
6.029
3.340
2.500
2.321
N
N
NH2
O
N
H
13
1.1
1.1
1.1
3.1
3.0
1.0
1.1
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1.1
3.4
ppm
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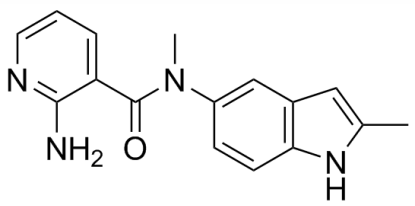
9
8
7
6
5
4
3
2
1
0




**13**

168.776
156.702
148.301
136.945
136.524
135.948
134.468
128.592
118.984
116.761
114.909
110.953
110.658
99.407
39.520
38.240
13.342

ppm 200 180 160 140 120 100 80 60 40 20 0


10.851
9.953
8.111
8.107
8.101
8.098
8.052
8.049
8.037
8.034
7.751
7.246
7.243
7.228
7.225
7.208
6.958
6.658
6.648
6.643
6.633
6.117
6.115
2.749
2.734
2.719
2.704
2.500
1.291
1.275
1.260
N
H
N
NH2
O
N
H
14
1.0
1.0
2.0
1.0
2.0
1.0
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0.9
3.0
ppm
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9
8
7
6
5
4
3
2
1
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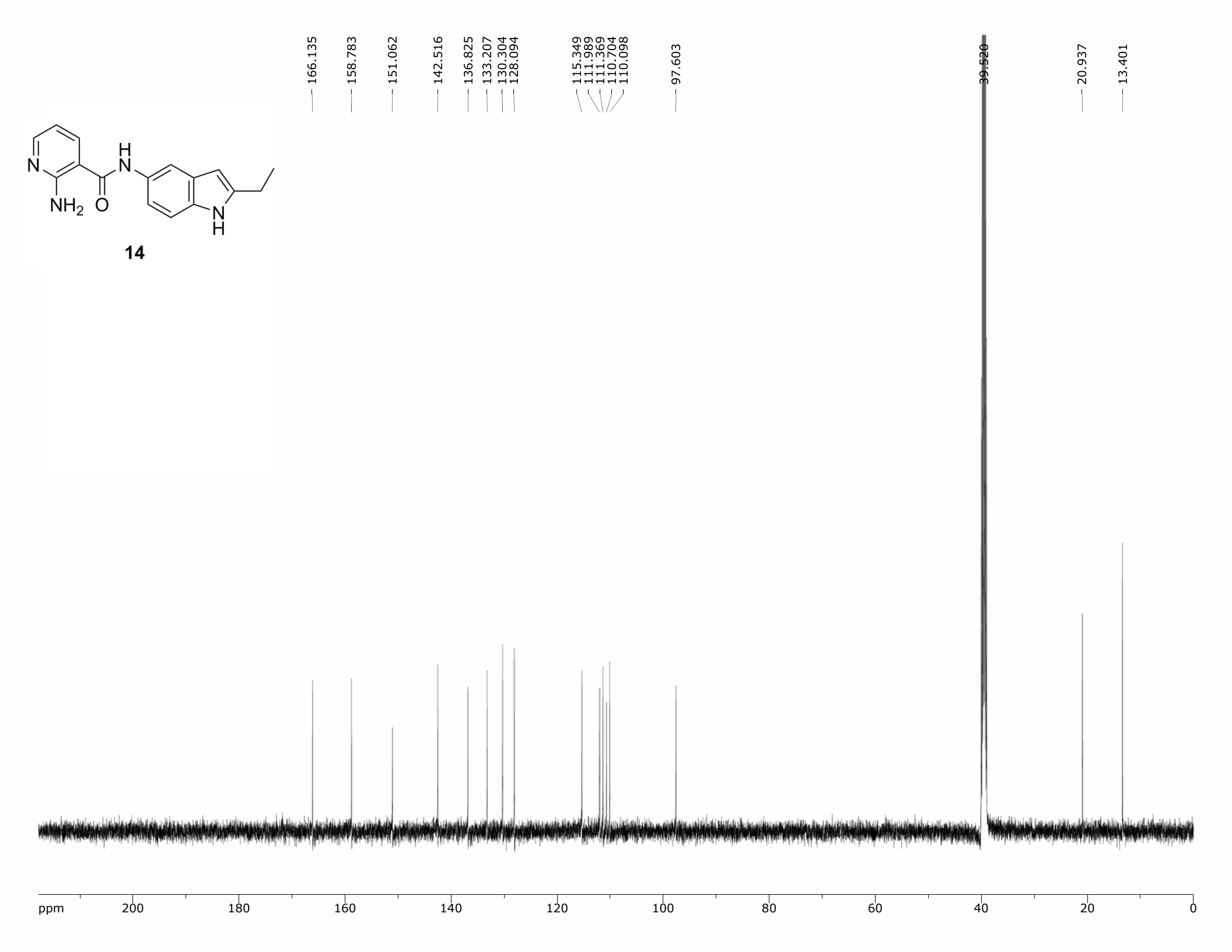
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130.304
128.094
115.349
111.989
111.369
110.704
110.098
97.603
39.520
20.937
13.401
N
H
N
NH2
O
N
H
14
ppm
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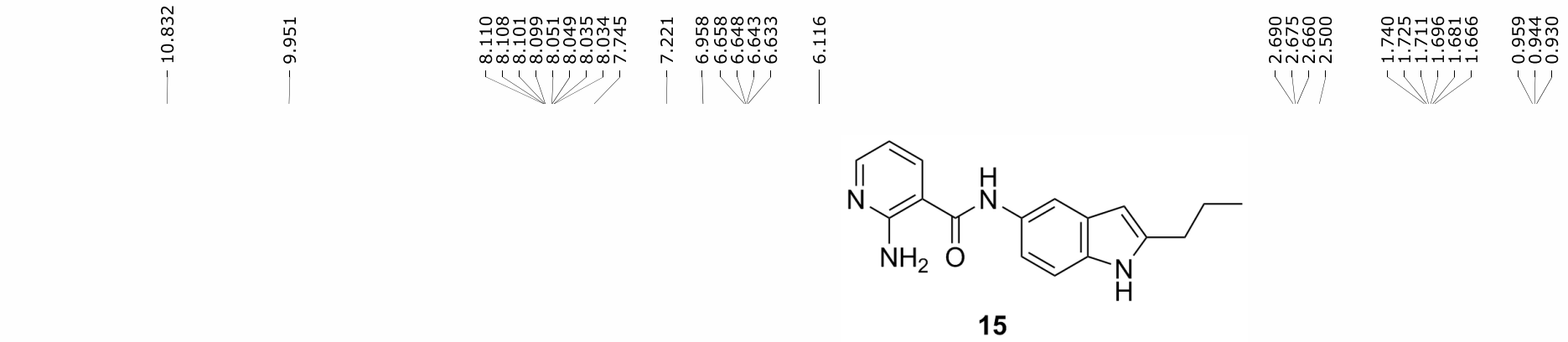
160
140
120
100
80
60
40
20
0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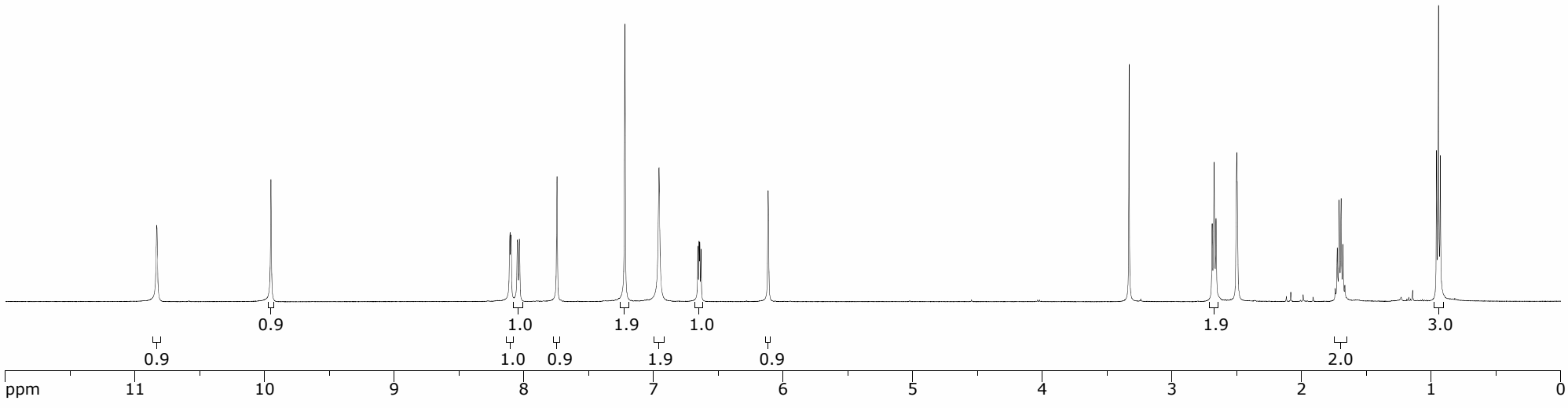
8.099
8.051
8.049
8.035
8.034
7.745
7.221
6.958
6.658
6.648
6.643
6.633
6.116
2.690
2.675
2.660
2.500
1.740
1.725
1.711
1.696
1.681
1.666
0.959
0.944
0.930
N
H
N
NH2
O
N
H

**15**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1.0
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3.0
0.9
1.0
0.9
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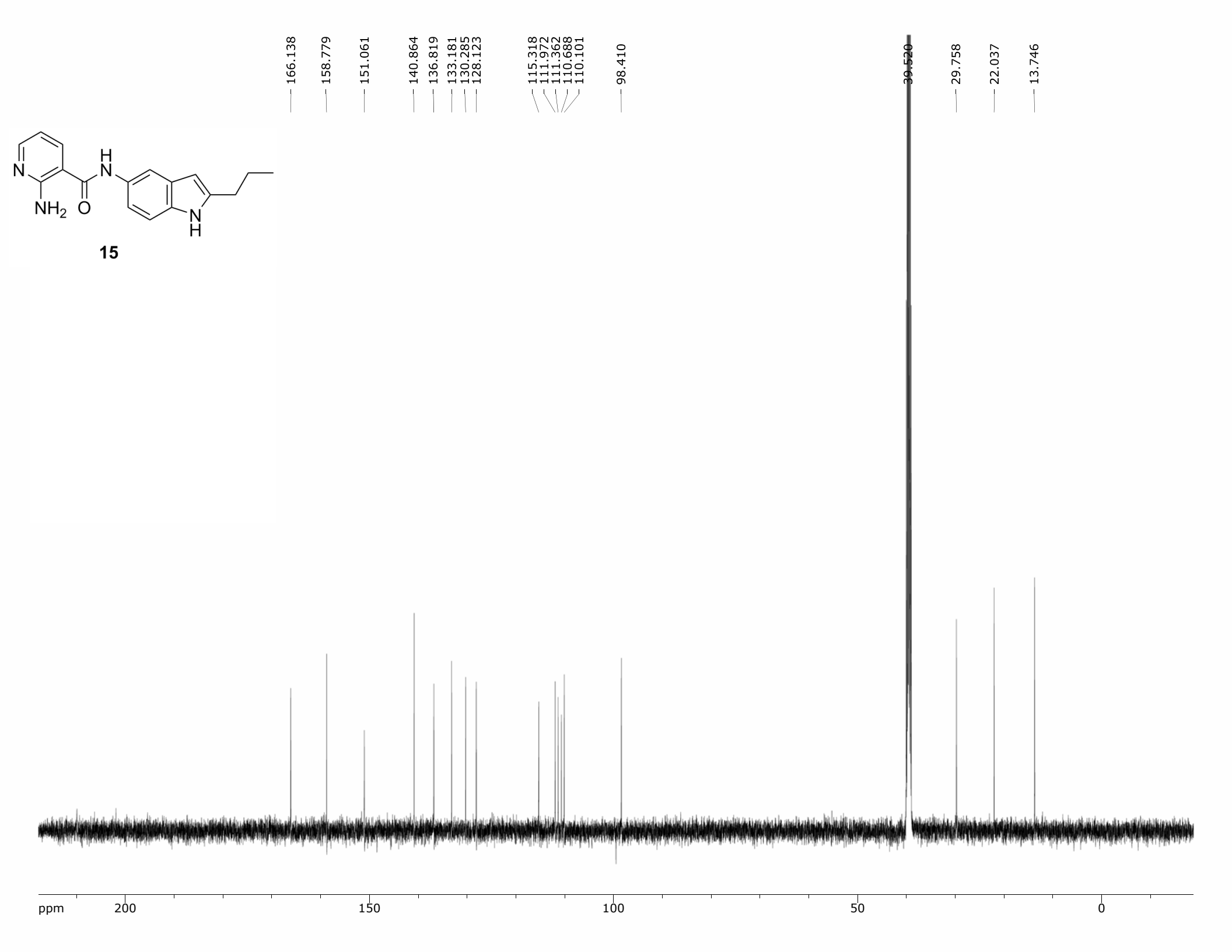
0.9
2.0
ppm
11
10
9
8
7
6
5
4
3
2
1
0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136.819
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130.285
128.123
115.318
111.972
111.362
110.688
110.101
98.410
39.520
29.758
22.037
13.746
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H
N
NH2
O
N
H
15
ppm
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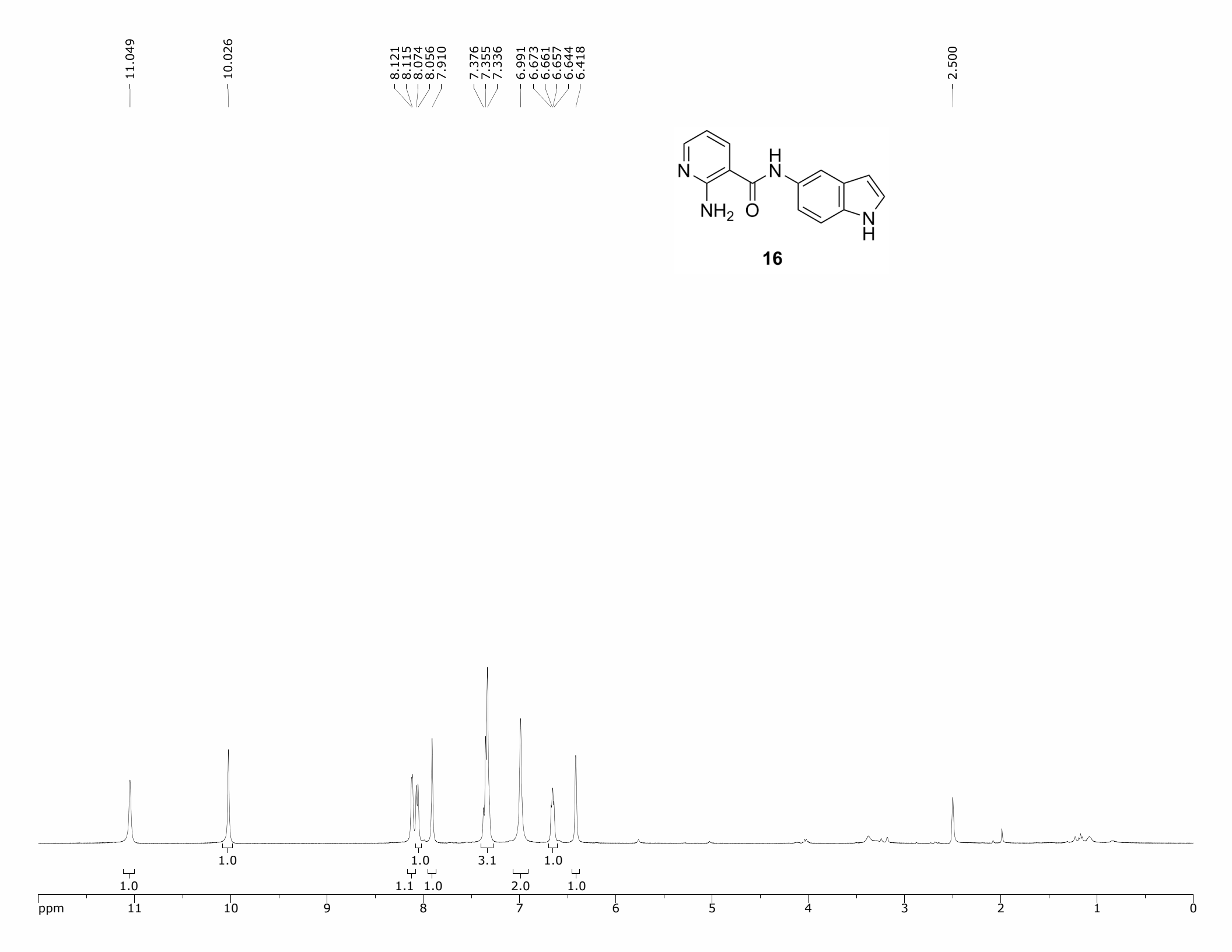
150
100
50
0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8.121
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8.056
7.910
7.376
7.355
7.336
6.991
6.673
6.661
6.657
6.644
6.418
2.500
N
H
N
NH2
O
N
H
16
1.0
1.0
3.1
1.0
1.0
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1.0
2.0
1.0
ppm
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10
9
8
7
6
5
4
3
2
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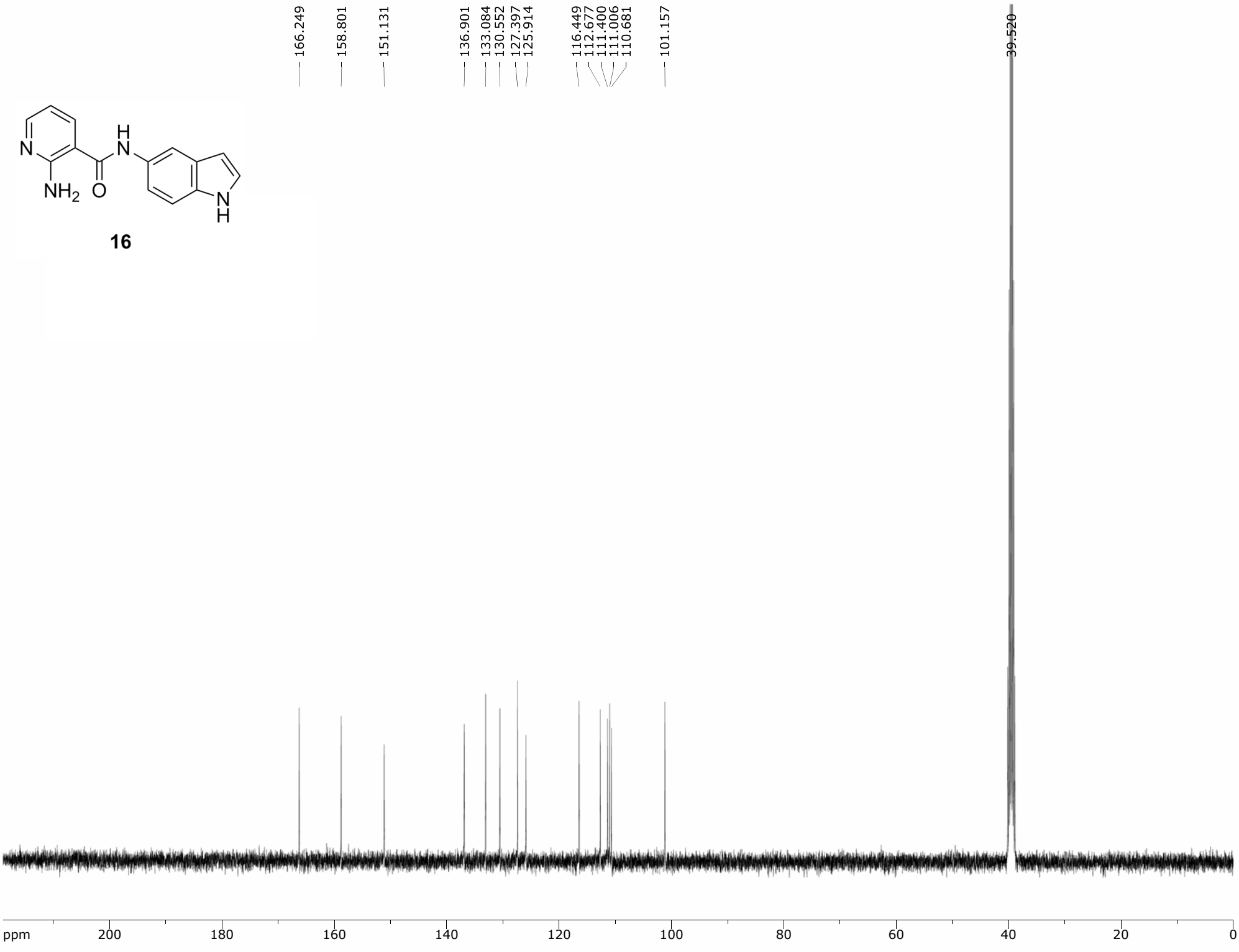
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130.552
127.397
125.914
116.449
112.677
111.400
111.006
110.681
101.157
39.520
N
H
N
NH2
O
N
H
16
ppm
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140
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40
20
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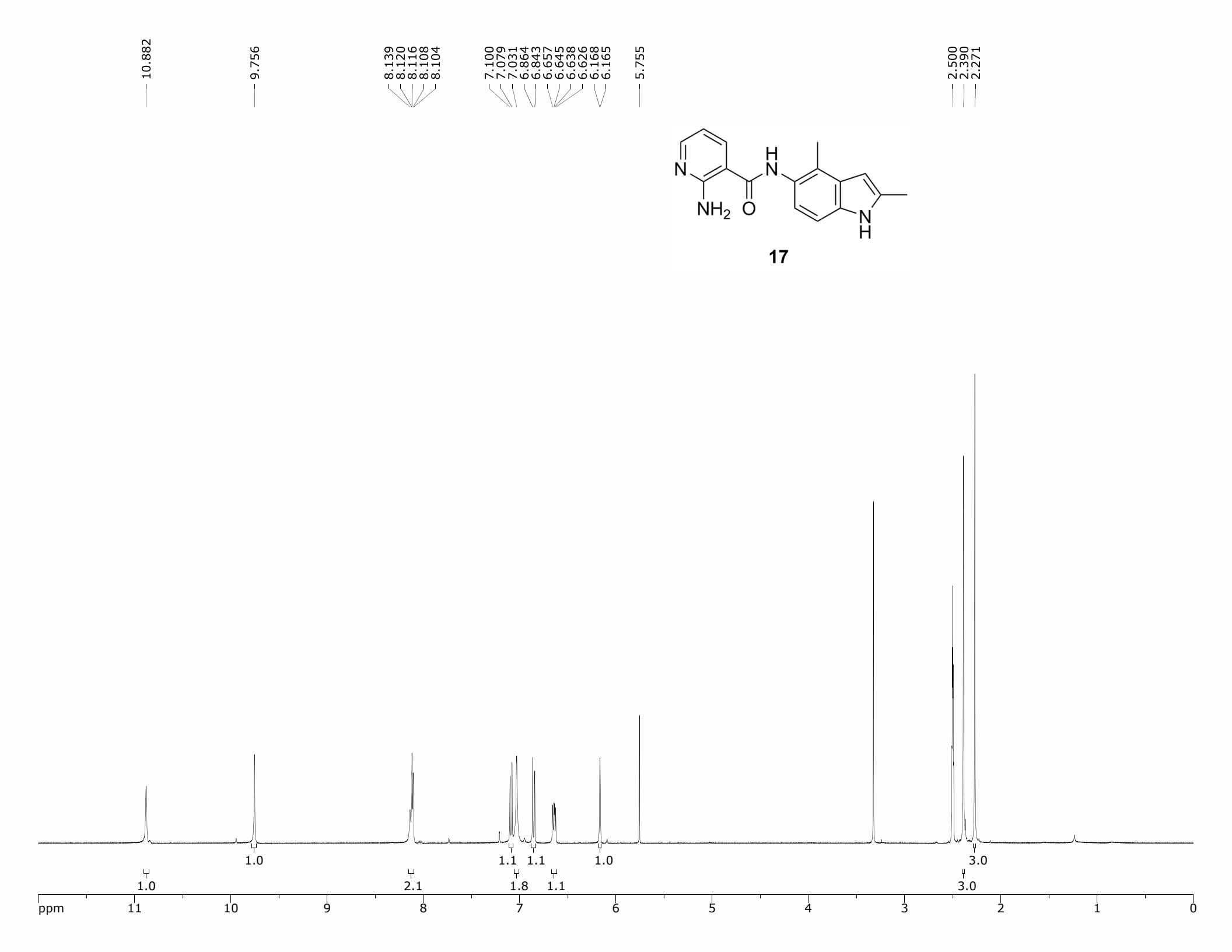
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7.100
7.079
7.031
6.864
6.843
6.657
6.645
6.638
6.626
6.168
6.165
5.755
2.500
2.390
2.271
N
H
N
NH2
O
N
H
17
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1.1
1.1
1.0
3.0
1.0
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3.0
ppm
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7
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1
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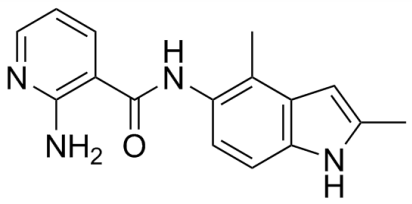


**17**

166.788
159.008
151.232
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135.430
134.065
129.000
126.519
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120.130
111.389
109.896
107.825
98.272
39.520
14.280
13.462

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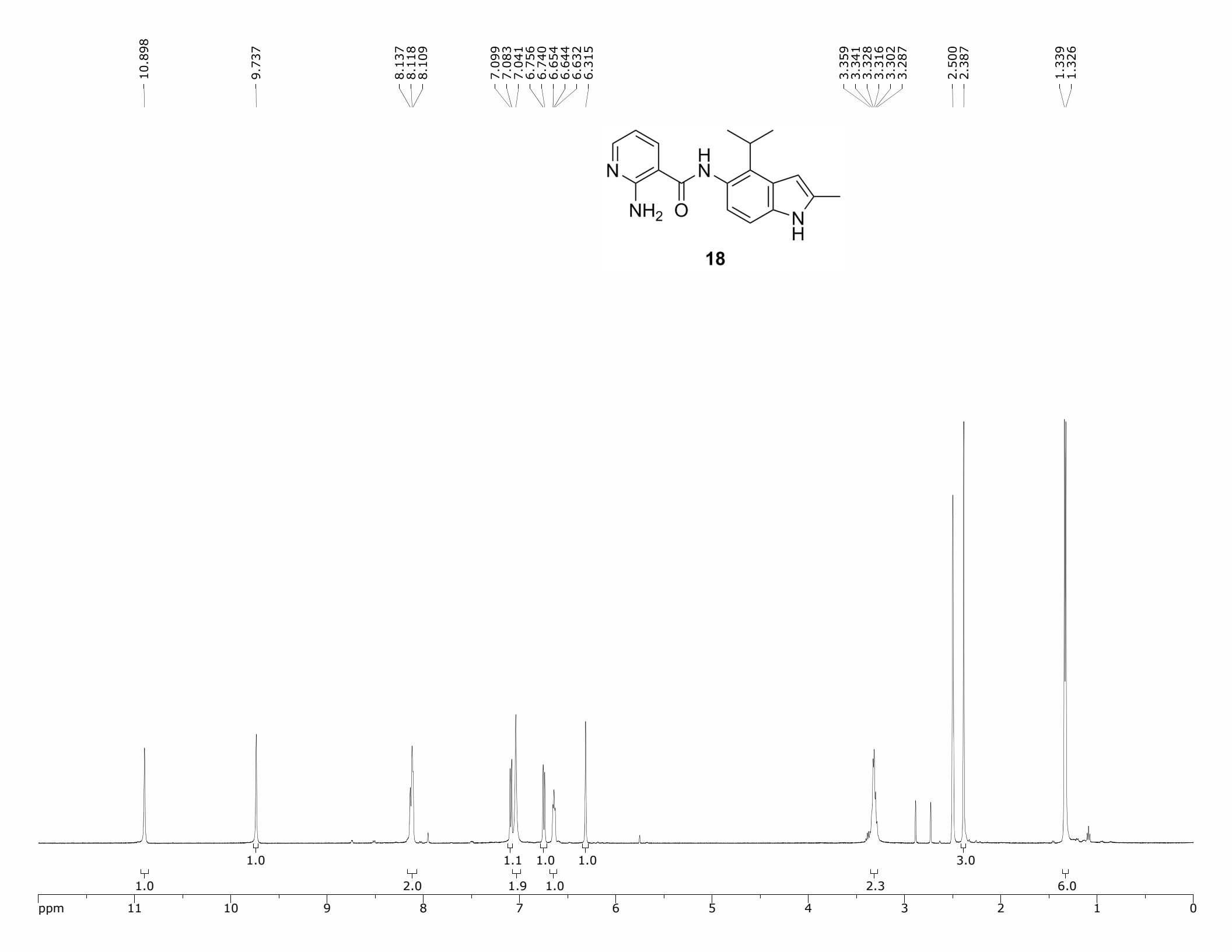
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8.109
7.099
7.083
7.041
6.756
6.740
6.654
6.644
6.632
6.315
3.359
3.341
3.328
3.316
3.302
3.287
2.500
2.387
1.339
1.326
N
H
N
NH2
O
N
H
18
1.0
1.1
1.0
1.0
3.0
1.0
2.0
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2.3
6.0
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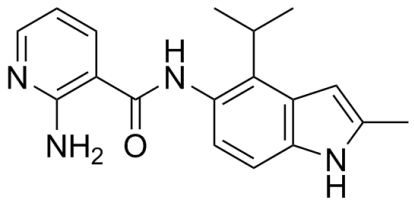
9
8
7
6
5
4
3
2
1
0


N
H
N
NH2
O
N
H

**18**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151.213
136.711
135.569
135.216
134.862
126.011
124.970
121.170
111.371
109.797
108.354
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13.371
ppm
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180
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60
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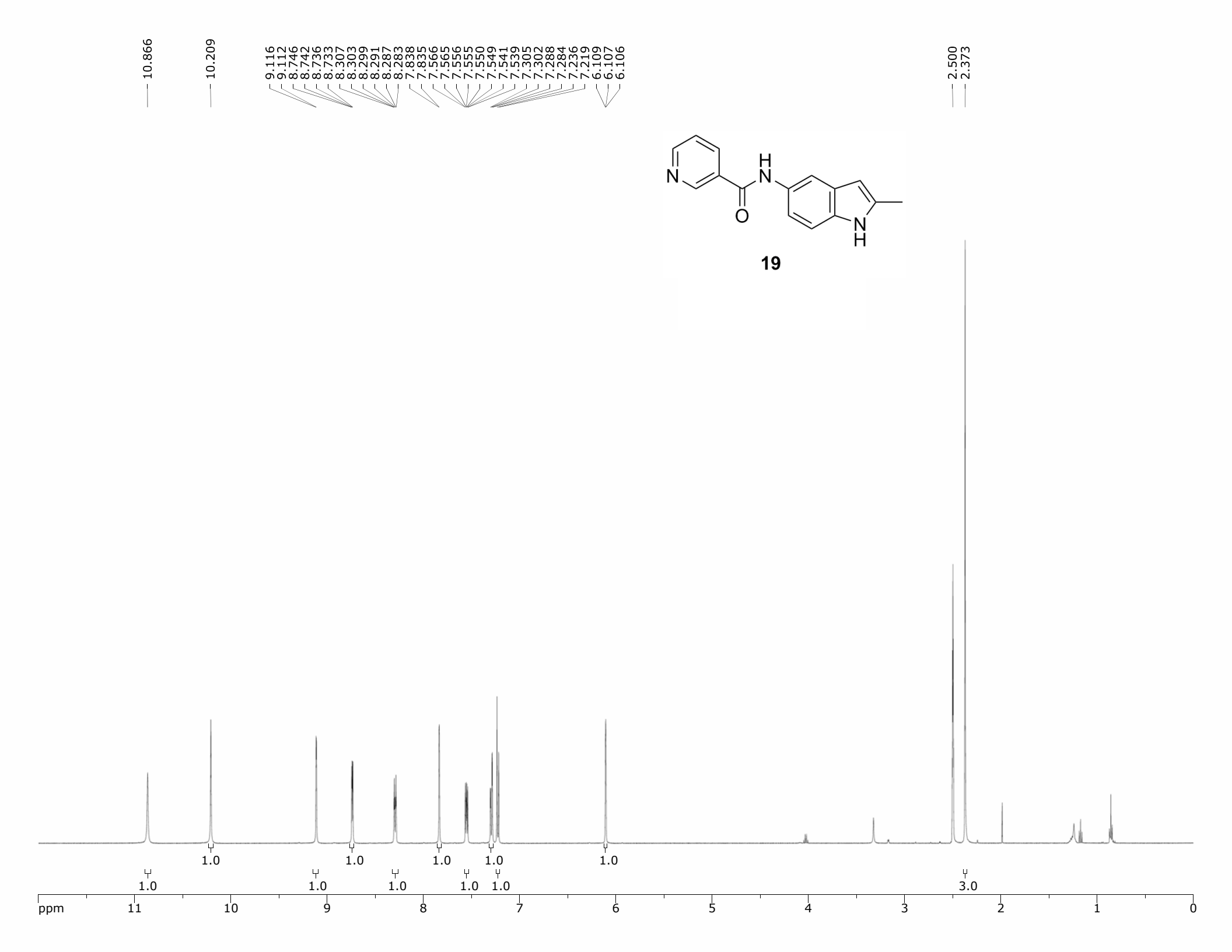
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9.112
8.746
8.742
8.736
8.733
8.307
8.303
8.299
8.291
8.287
8.283
7.838
7.835
7.566
7.565
7.556
7.555
7.550
7.549
7.541
7.539
7.305
7.302
7.288
7.284
7.236
7.219
6.109
6.107
6.106
2.500
2.373
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H
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1.0
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3.0
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7
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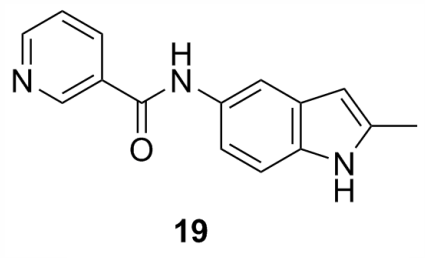
4
3
2
1
0



**19**

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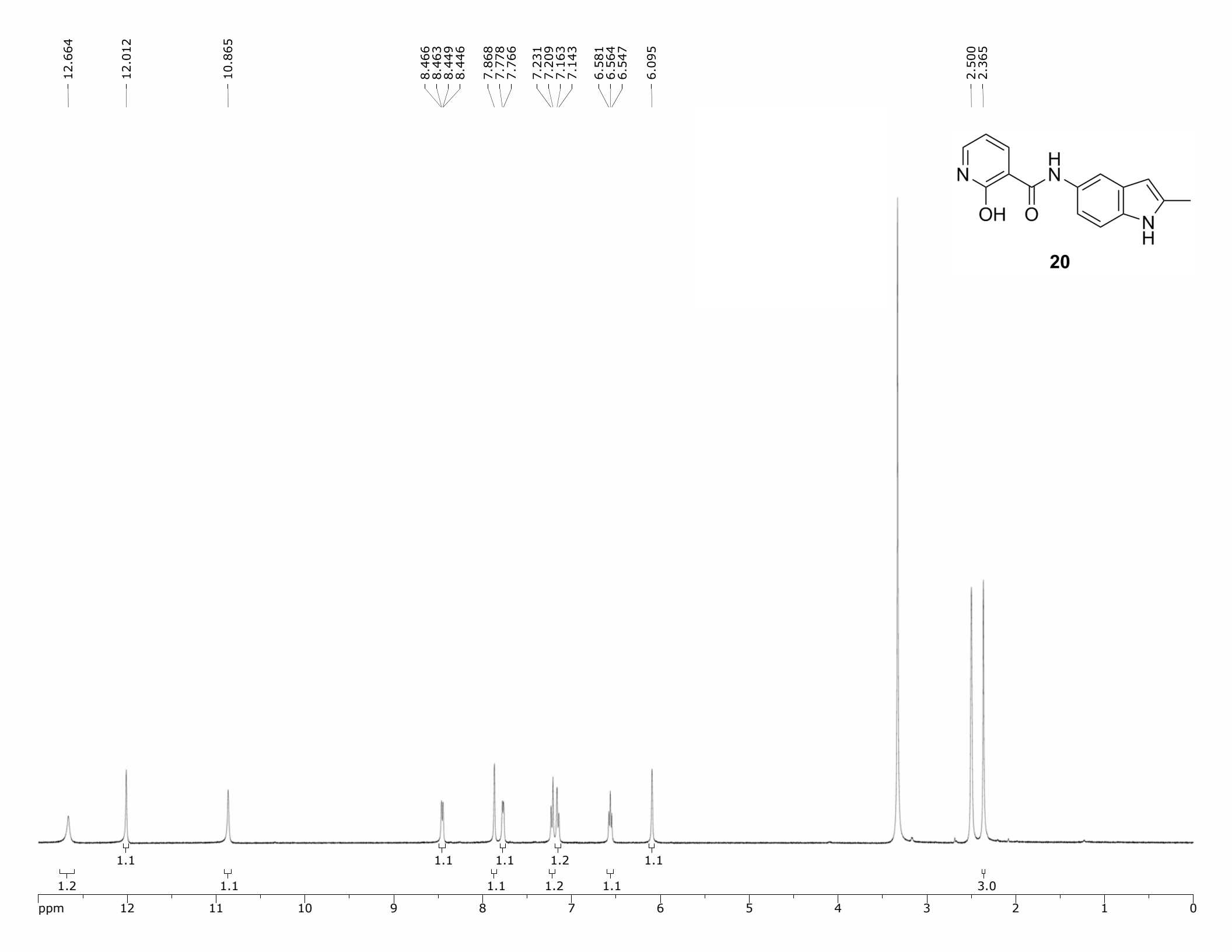
151.683
148.541
136.312
135.215
133.290
131.012
130.377
128.364
123.385
114.743
111.347
110.084
99.252
39.520
13.405

ppm 200 180 160 140 120 100 80 60 40 20 0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8.449
8.446
7.868
7.778
7.766
7.231
7.209
7.163
7.143
6.581
6.564
6.547
6.095
2.500
2.365
N
H
N
OH
O
N
H
20
1.1
1.1
1.1
1.2
1.1
1.2
1.1
1.1
3.0
ppm
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11
10
9
8
7
6
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4
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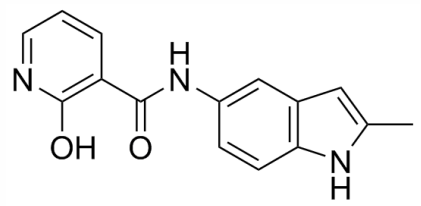
2
1
0


N
H
N
OH
O
N
H

**20**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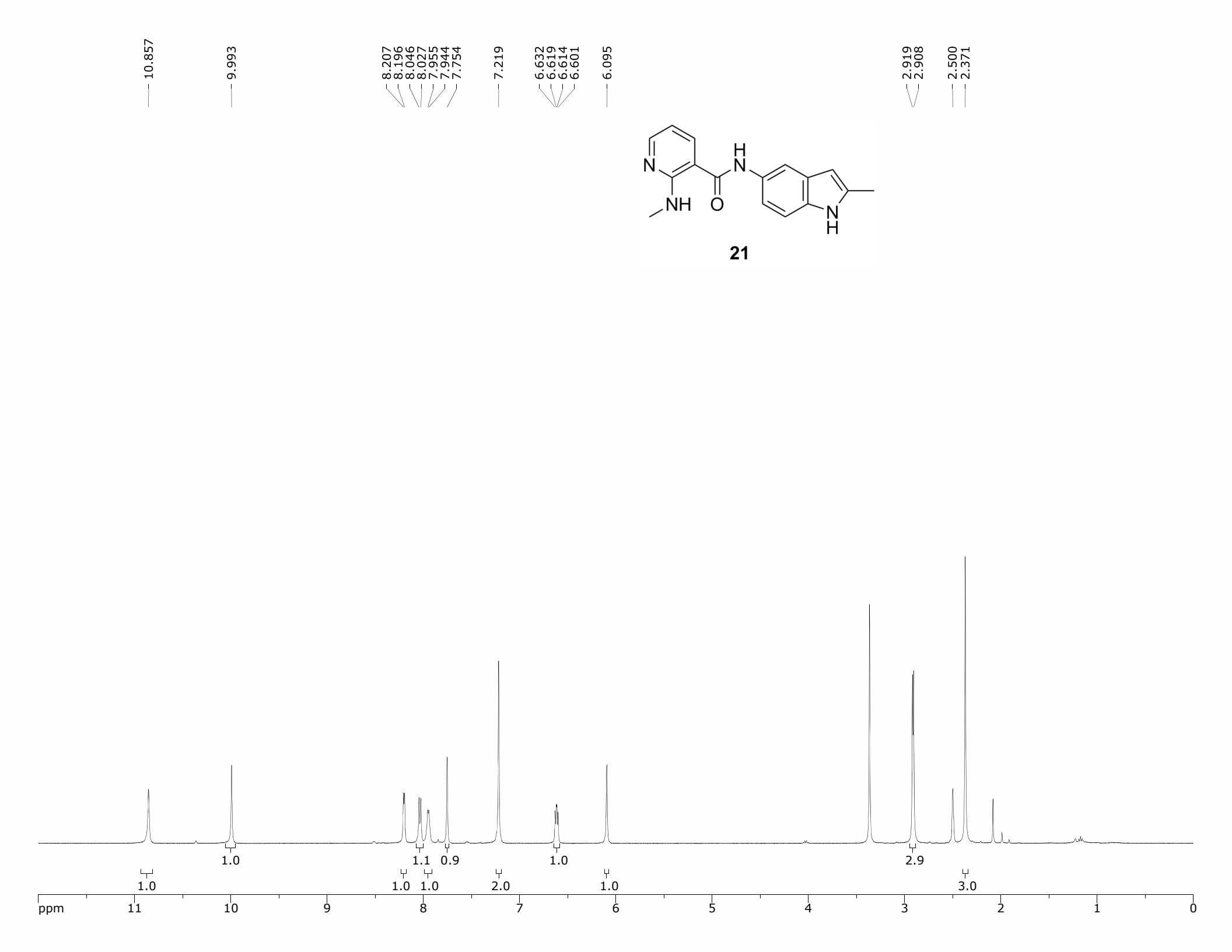
160.826
144.000
139.468
136.477
133.138
130.366
128.703
120.704
113.671
110.552
110.063
106.788
99.319
39.520
13.441
ppm
200
180
160
140
120
100
80
60
40
20
0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8.046
8.027
7.955
7.944
7.754
7.219
6.632
6.619
6.614
6.601
6.095
2.919
2.908
2.500
2.371
N
H
N
NH
O
N
H
21
1.0
1.1
0.9
1.0
2.9
1.0
1.0
1.0
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1.0
3.0
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10
9
8
7
6
5
4
3
2
1
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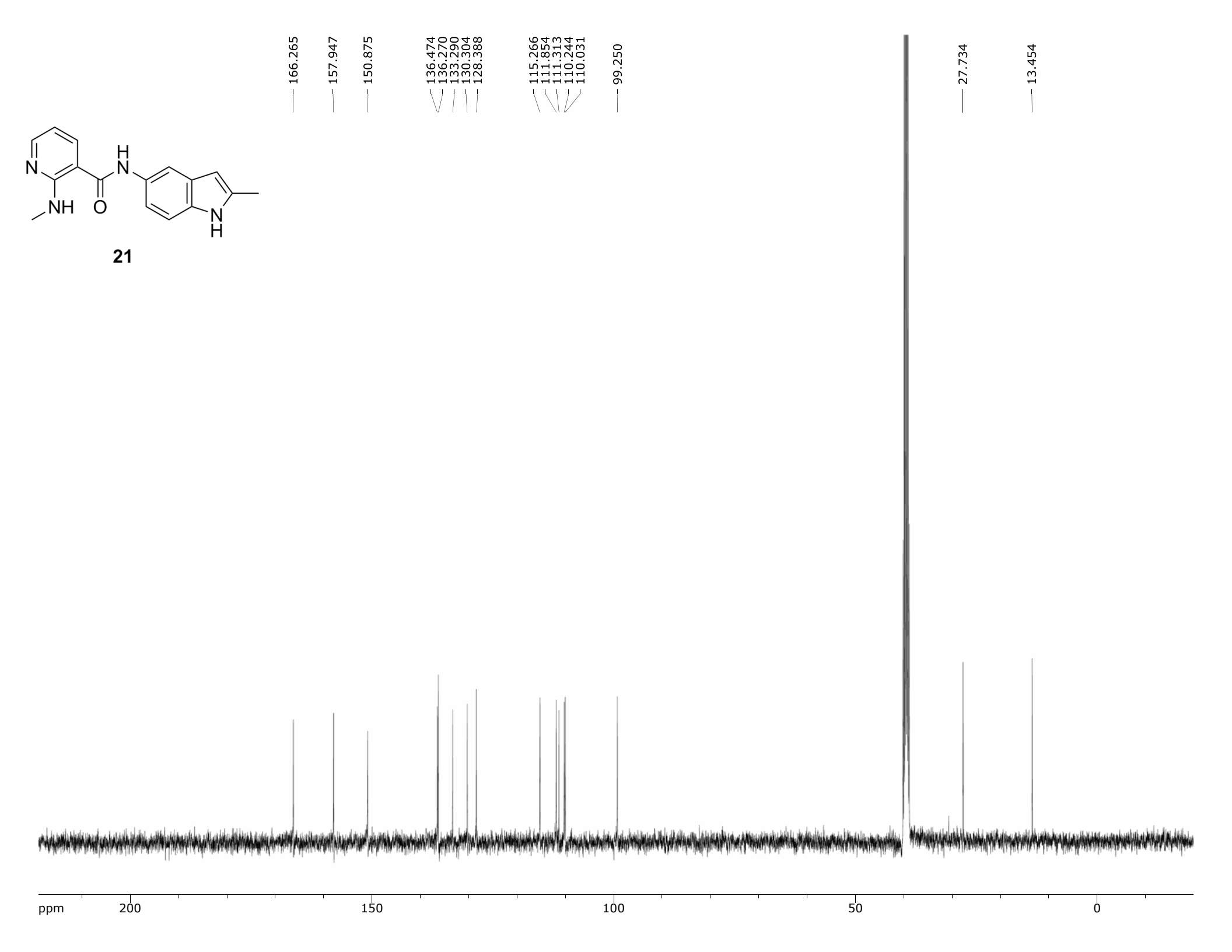
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99.250
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13.454
N
H
N
NH
O
N
H
21
ppm
200
150
100
50
0